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HÉPATECTOMIE PARTIELLE ET RÉSISTANCE AUX BRÛLURES

V. VARIATIONS DE L'ACIDE ASCORBIQUE ET DU POIDS DES SURRÉNALES [1]

PAR L. P. DUGAL ET A. DESMARAIS

Abstract

The changes in adrenal ascorbic acid and weight have been studied in four groups of animals: (1) partially (65%) hepatectomized rats, (2) partially hepatectomized ones, burned six hours after operation, (3) sham operated controls, and (4) sham operated controls burned six hours after operation. Results show slight but significant differences in ascorbic acid concentration and adrenal weight between Group 2, in which the mortality is higher, and the other groups: ascorbic acid concentration is a little lower in the first 24 hr. after burns, and adrenal weight becomes higher only 48 hr. after burning.

Dans des travaux précédents, nous avons montré que l'hépatectomie partielle diminue la résistance du rat blanc aux brûlures (4). Afin de trouver une explication à ce phénomène, nous avons entrepris d'étudier la régénération du foie après les brûlures (5), l'influence du jeûne sur la mortalité des animaux partiellement hépatectomisés et brûlés (article sous presse), les variations de la glycémie (2 et article sous presse) et de l'azote-α-aminé du sang total (3).

Le présent travail est consacré à l'étude des variations de l'acide ascorbique et du poids des surrénales chez des rats blancs partiellement hépatectomisés et leurs témoins opérés à blanc, brûlés et non brûlés.

Protocole expérimental

Des lots de rats albinos, tous mâles et de poids équivalents (125 à 145 grammes) ont été divisés en quatre groupes: (1) témoins opérés à blanc, non brûlés; (2) témoins opérés à blanc et brûlés; (3) hépatectomisés (65%), non brûlés; (4) hépatectomisés (65%) et brûlés. Six heures après les opérations, les animaux des groupes 2 et 4 furent brûlés suivant une méthode standard que nous avons déjà décrite (4).

L'extraction et les dosages de l'acide ascorbique ont été effectués par la méthode de Bessey et King (1), à des intervalles de 0, 6, 12, 24, 48, 72, 96 et 120 heures après les brûlures, et à des périodes correspondantes pour les non

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brûlés. Les surrénales, prélevées sous anesthésie à l'éther, étaient d'abord déposées sur un papier filtre, soigneusement débarrassées du tissu adipeux adhérent, pesées, puis déposées dans le mélange d'extraction. L'ensemble de ces opérations ne dure pas plus que quatre minutes.

Les résultats que nous allons exposer sont basés sur des moyennes obtenues des dosages et pesées effectués sur les surrénales de 10 animaux au moins, soit, pour toute l'expérience, près de 400 animaux.

Résultats

(1) *Acide ascorbique* (mg. par gr. de tissu frais)

La concentration normale de l'acide ascorbique dans les surrénales, chez les animaux de 135 à 145 grammes, est de 4.60 ± 0.34 mg.

Les résultats obtenus apparaissent dans le tableau ci-joint. Nous voyons que la concentration diminue au cours des six heures qui suivent l'opération: l'abaissement est de 35.8% de la valeur normale chez les témoins et de 33.4% chez les hépatectomisés. Au moment des brûlures (point 0), hépatectomisés et témoins ont donc des concentrations d'acide ascorbique sensiblement identiques.

TABLEAU I

I. Acide ascorbique (mg. par gr. de tissu frais). Normale: $4.60 \pm 0.34^*$
II. Poids des surrénales (en mg.). Normale: 14.6 ± 1.5

Heures après les brûlures	Témoins non brûlés				Témoins brûlés			
	I		II		I		II	
0	2.95** ±	0.19	24.9 ±	1.7	2.95** ±	0.19	24.9 ±	1.7
6	3.85 ±	0.28	25.5 ±	3.0	3.31 ±	0.30	29.3 ±	2.3
12	3.80 ±	0.30	29.6 ±	2.4	3.45 ±	0.29	31.6 ±	2.4
24	3.67 ±	0.26	27.5 ±	2.3	2.86** ±	0.26	29.0 ±	2.4
48	4.03 ±	0.34	28.1 ±	2.1	4.78 ±	0.41	32.1 ±	2.4
72	4.12 ±	0.36	29.4 ±	2.2	3.54 ±	0.30	33.1 ±	2.6
96	3.88 ±	0.32	28.3 ±	3.1	4.04 ±	0.32	32.6 ±	2.6
120	4.04 ±	0.33	30.1 ±	2.2	4.14 ±	0.34	34.3 ±	2.7
	Hépatectomisés non brûlés				Hépatectomisés brûlés			
	I		II		I		II	
0	3.06** ±	0.21	23.9 ±	1.7	3.06** ±	0.21	23.9 ±	1.7
6	3.69 ±	0.38	30.3 ±	2.4	2.93** ±	0.28	29.8 ±	2.3
12	3.58 ±	0.29	29.8 ±	2.4	2.77** ±	0.26	32.2 ±	2.6
24	3.33 ±	0.30	32.3 ±	2.4	3.11** ±	0.27	28.7 ±	2.2
48	4.48 ±	0.36	29.7 ±	2.2	4.25 ±	0.34	29.6 ±	2.3
72	4.25 ±	0.33	30.7 ±	2.2	3.85 ±	0.31	37.3 ±	3.2
96	4.27 ±	0.31	31.7 ±	2.3	3.84 ±	0.29	35.4 ±	2.9
120	3.89 ±	0.29	32.8 ±	2.4	3.72 ±	0.25	41.5 ±	3.0

* *Erreur probable.*
** *Ecarts de la normale statistiquement significatifs.*

A partir du point 0, la concentration augmente chez tous les groupes, sauf les hépatectomisés brûlés où l'abaissement se continue jusqu'à 12 heures après les brûlures, pour atteindre une valeur minima de 39.7%. Après 120 heures (cinq jours), les valeurs se sont progressivement rapprochées de la normale chez tous les groupes.

L'analyse statistique de ces résultats nous a montré que les moyennes obtenues sont très significatives; les valeurs de "*t*" sont toutes comprises entre 2.2 et 6.8, ce qui indique une probabilité de 95% et plus. Les différences entre les groupes, considérées à quelque moment que ce soit après les brûlures, sont peu considérables et n'ont pas de valeur statistique.

Il est intéressant de noter que le groupe des hépatectomisés brûlés est le seul chez lequel toutes les valeurs de la concentration, au cours des 24 premières heures après les brûlures, ont un écart de la normale statistiquement significatif.

Nos résultats, chez les animaux brûlés, confirment ceux de Ludewig (8) et de Long (6, 7).

(2) *Poids des surrénales*

Le tableau ci-joint (Tableau I) donne aussi les variations du poids des surrénales, exprimées en mg., chez ces mêmes animaux. Le poids de ces organes chez les animaux normaux témoins est de 14.6 ± 1.5 mg.

Nous constatons, dans les six heures qui suivent les opérations, une forte augmentation du poids des surrénales, à peu près identique chez les témoins et chez les hépatectomisés, soit 63.7% pour ces derniers et 70.1% pour les premiers. Après les brûlures, l'augmentation est moins rapide; à la fin de la période expérimentale, nous avons les valeurs suivantes, exprimant le poids acquis depuis le moment des brûlures (point 0):

Témoins non brûlés............................ 37.2%

Témoins brûlés................................ 37.7%

Hépatectomisés non brûlés..................... 20.9%

Hépatectomisés brûlés......................... 73.6%

Cette augmentation, qui dissocie des trois autres groupes celui des hépatectomisés brûlés, est acquise de 48 à 120 heures après les brûlures. Avant 48 heures, l'évolution est la même dans les quatre groupes, et c'est la période importante, puisque, nous l'avons déjà montré (4), c'est au cours des 48 premières heures après les brûlures que la mortalité est la plus forte chez les hépatectomisés brûlés.

Interprétation

Alors que nous nous attendions à des différences considérables dans le niveau de l'acide ascorbique et le poids des surrénales entre les hépatectomisés brûlés et les autres groupes, l'ensemble des résultats montre qu'il n'en est rien. Les seules différences observées entre les hépatectomisés brûlés et les trois autres groupes sont les suivantes: un abaissement un peu plus marqué et

significatif de la concentration en acide ascorbique au cours des 24 premières heures après les brûlures; une plus grande augmentation du poids des sur-rénales, se produisant seulement 48 heures après les brûlures.   Ces différences ne nous permettent pas d'apporter à notre problème une solution définitive.

L'ensemble de nos résultats soulève la question de la relation entre l'intensité du "stress" et celle de la réponse des surrénales.   De plus, il est nécessaire de noter que, chez les hépatectomisés brûlés, la survie s'améliore à mesure que progresse la régénération hépatique.   Si nous empruntons à nos travaux précédents les chiffres obtenus sur la survie des hépatectomisés (4) et la régénération du foie (5) après les brûlures, nous obtenons le tableau suivant, montrant que la mortalité régresse à mesure que le foie régénère.

Période, heures	Mortalité, %	Régénération, %
0 – 24	24.0	1.3
24 – 48	11.0	19.5
48 – 72	9.0	17.6
72 – 96	9.0	4.3
96 – 120	1.0	8.3

Tout se passe comme si l'intégrité de la fonction hépatique était nécessaire, soit pour assurer la capacité réactionnelle des tissus à la sécrétion corticale, soit pour activer les hormones corticales.   Les récents travaux de Roberts et Szego (9) ont montré que le foie joue un rôle dans l'activation des oestrogènes, probablement en assurant la formation d'un composé soluble dans l'eau entre ces substances et une protéine d'origine hépatique.   Le foie ne pourrait-il jouer un rôle semblable dans l'activation des stéroïdes corticaux?   C'est l'hypothèse qui ressort de nos travaux sur l'hépatectomie partielle et la résist-ance aux brûlures.   Les expériences en cours nous permettront de vérifier si une telle hypothèse est fondée, et peur-être d'éclairer d'un jour nouveau les relations entre la fonction hépatique et la réaction surrénalienne d'adaptation.

Bibliographie

1. BESSEY, O. A. et KING, C. G.   J. Biol. Chem. 103 : 687.   1933.
2. DesMARAIS, A. et DUGAL, L. P.   Rev. can. biol. 6(2) : 368.   1947.
3. DesMARAIS, A. et DUGAL, L. P.   Rev. can. biol. 7(2) : 207.   1948.
4. DUGAL, L. P., DesMARAIS, A., DESAULNIERS, L., ET RINFRET, M.   Rev. can. biol. 3(4) : 474.   1944.
5. DUGAL, L. P. et DesMARAIS, A.   Rev. can. biol. 7(2) : 212.   1948.
6. LONG, C. N. H.   Federation Proc. 6(2) : 461.   1947.
7. LONG, C. N. H.   Recent progress in hormone research.   Vol. 1, pp. 123 à 145.   Academic Press, Inc., New York.   1947.
8. LUDEWIG, S. et CHANUTIN, A.   Endocrinology, 41(2) : 135.   1947.
9. ROBERTS, S. et SZEGO, C. M.   Endocrinology, 40 : 73.   1947.

LIPIDS OF THE NERVOUS SYSTEM DURING
IN VITRO DEGENERATION[1]

By A. C. JOHNSON, A. R. MCNABB, AND R. J. ROSSITER

Abstract

The changes in the concentration of lipids during the *in vitro* degeneration of cat brain slices and sciatic nerve have been determined after incubation in bicarbonate buffer for periods of time from 1 to 14 days. In brain slices incubated for 14 days there was a great decrease in the concentration of total phospholipid with no change in the concentration of cerebroside or total cholesterol. Of the individual phospholipids, there was a decrease in the concentration of sphingomyelin and cephalin and no significant change in the concentration of lecithin. In sciatic nerve incubated for eight days, there was a significant decrease in the concentration of cephalin and a decrease of borderline significance in the concentration of total phospholipid. There was no significant change in the concentration of cerebroside, free or total cholesterol, sphingomyelin, or lecithin. Thus in tissue of the nervous system, there appears to be a phospholipid-splitting mechanism, possibly enzymatic in nature.

Introduction

In previous reports on the changes in lipid concentration during the *in vitro* autolysis of tissue from the nervous system, brain only was used and only a limited number of the lipid components were estimated. In this investigation, the concentration of cerebroside, free and total cholesterol, total phospholipid, lecithin, sphingomyelin, and cephalin was determined during *in vitro* degeneration of both brain slices and peripheral nerve.

Changes in lipid content of nerves degenerating *in vivo* have been reported by Mott and Halliburton (16), May (14), Falk (4), Randall (19), and Johnson, McNabb, and Rossiter (8). Investigations on the lipids of cat sciatic nerve during Wallerian degeneration (Johnson *et al.* (8)) seemed incomplete without carrying out parallel *in vitro* studies. Monckeberg and Bethe (15), Nageotte (17), and Cajal (2) showed that the histological changes of nerves autolyzing *in vitro* were typical of those found in Wallerian degeneration.

As far as we know, there are no reports in the literature on the lipid content of autolyzed nerve. However, there are some data on the concentration of lipids of brain tissue during autolysis. Simon (21), Stamm (24), and Fries, Schachner, and Chaikoff (5) reported a hydrolysis of phospholipids during incubation. The most recent work is that of Sperry (22), who found a constant decrease in the concentration of total phospholipid in homogenates of rat brain. Jungmann and Kimmelstiel (10) reported that cerebroside decreased and inorganic phosphorus increased in autolyzing rabbit brain. None of the above workers estimated the individual phospholipids.

Methods

Autolysis of Brain

The brain of a cat was removed as soon as possible after death. With aseptic precautions brain slices were cut from each frontal lobe, one side providing the tissue for the control, the other side the sample for autolysis. The slices were transferred to two tared sterile Thunberg tubes, each containing 3 ml. of either Krebs bicarbonate buffer, pH 7.4, or bicarbonate buffer with isotonic saline. The Krebs bicarbonate buffer contained the cations, sodium, potassium, calcium, and magnesium, and the anions, chloride and phosphate, in approximately physiological proportions, while the only ions, other than bicarbonate, present in the bicarbonate buffer with isotonic saline were sodium and chloride. To each tube was added as a preservative sufficient merthiolate to give a final concentration of 1/5000. After reweighing, the Thunberg tubes were gassed with a mixture of either 5% carbon dioxide in nitrogen or 5% carbon dioxide in oxygen. The tube containing the sample to be incubated was placed in a 37° C. water bath for periods of time varying from 1 to 14 days. Immediately after gassing, the brain slices in the control tube were removed and extracted with a 1 : 1 ethyl alcohol – ether mixture as outlined previously (Johnson, McNabb, and Rossiter (6)).

The tubes were not regassed during incubation and after 14 days the pH, determined by glass electrode, had not changed. After incubation, a sample of the medium was taken for bacterial culture, the slices removed, and the lipids extracted as described for the controls.

Autolysis of Nerve

Both sciatic nerves of a cat were removed with aseptic precautions and treated in a similar manner to the brain slices. The nerve to be incubated was transferred to a Thunberg tube containing Krebs bicarbonate buffer and gassed with a mixture of 5% carbon dioxide in nitrogen. The tube was placed in a water bath at 37° C. for eight days. An incubation period of eight days was selected for it corresponded to one of the intervals used in the experiments on degeneration of nerves *in vivo* (Johnson *et al.* (8)).

Estimation of Lipids

The methods adopted for the estimation of the lipids were the same as those described previously (Johnson *et al.* (6)). The concentration of cerebroside, free and total cholesterol, total phospholipid, monoaminophosphatide, and lecithin was determined and from these figures was calculated the concentration of ester cholesterol, sphingomyelin, and cephalin. It has been shown that the coefficient of variation of the over-all procedures were as follows: cerebroside $\pm$ 3.5%, free cholesterol $\pm$ 0.9%, total cholesterol $\pm$ 0.9%, total phospholipid $\pm$1.3%, monoaminophosphatide $\pm$ 1.6%, lecithin $\pm$ 5.1%, sphingomyelin $\pm$ 11.9%, and cephalin $\pm$ 3.9%. Since

the estimations on brain were done in triplicate and those on nerve in duplicate, the probable error of each observation was, therefore, less than the above figure.

Results

The results were recorded as the difference between the concentration of each individual lipid in the control sample and the concentration of the same lipid in the incubated sample, expressed as a percentage of the control.   Five or six experiments were done in each group and the mean and standard error of the mean presented for each lipid (Tables I to IV).   The variation from

TABLE I

MEAN PERCENTAGE CHANGE IN LIPIDS OF CAT BRAIN, INCUBATED 24 HR.
IN CO_2-N_2 MIXTURE (FIVE EXPERIMENTS)

	Bicarbonate buffer with saline			Krebs bicarbonate buffer		
	Mean change (%)	S.E.M.	P	Mean change (%)	S.E.M.	P
Cerebroside	+ 0.28	± 5.70	>0.9	−10.30	±4.26	>0.05
Free cholesterol	+ 5.38	± 7.35	>0.4	+ 0.72	±2.11	>0.6
Total cholesterol	+ 4.52	± 6.54	>0.5	− 0.10	±3.01	>0.9
Total phospholipid	+ 7.30	± 9.80	>0.5	− 7.50	±4.55	>0.1
Lecithin	−18.70	±15.54	>0.2	+ 2.68	±1.18	>0.05
Sphingomyelin	+ 0.34	±16.24	>0.9	− 1.90	±9.65	>0.8
Cephalin	− 3.02	±10.90	>0.7	−13.10	±4.44	<0.02

TABLE II

MEAN PERCENTAGE CHANGE IN LIPIDS OF CAT BRAIN, INCUBATED SEVEN DAYS
IN KREBS BICARBONATE BUFFER (FIVE EXPERIMENTS)

	CO_2/N_2			CO_2/O_2		
	Mean change (%)	S.E.M.	P	Mean change (%)	S.E.M.	P
Cerebroside	+ 4.02	± 8.00	>0.1	− 1.80	±19.05	>0.9
Free cholesterol	− 7.08	± 7.25	>0.2	− 1.40	± 3.51	>0.7
Total cholesterol	− 6.90	± 6.93	>0.3	− 0.30	± 3.97	>0.9
Total phospholipid	−14.38	± 4.74	<0.05	−30.30	± 5.44	<0.01
Lecithin	+ 6.20	± 7.05	>0.4	−13.26	±12.78	>0.3
Sphingomyelin	− 8.50	±20.95	>0.7	−48.72	±13.54	<0.01
Cephalin	−29.82	± 6.45	<0.02	−36.24	± 5.32	<0.01

experiment to experiment was great.   This is brought out in the tables by the high value of the standard error of the mean.

There was no significant change in the concentration of lipids of brain slices incubated for 24 hr. in bicarbonate buffer with normal saline (Table I).

TABLE III

MEAN PERCENTAGE CHANGE IN LIPIDS OF CAT BRAIN, INCUBATED 14 DAYS
IN KREBS BICARBONATE BUFFER (CO_2-N_2 MIXTURE)

	No. observations	Mean change (%)	S.E.M.	P
Cerebroside	5	− 5.16	±18.15	>0.8
Free cholesterol	6	+ 1.38	± 2.65	>0.6
Total cholesterol	6	+ 3.44	± 2.08	>0.1
Total phospholipid	6	−52.66	±10.20	<0.01
Lecithin	4	−18.90	±16.76	>0.3
Sphingomyelin	5	−58.40	± 9.40	<0.01
Cephalin	4	−46.00	±10.28	<0.05

TABLE IV

MEAN PERCENTAGE CHANGE IN LIPIDS OF CAT SCIATIC NERVE, INCUBATED EIGHT
DAYS IN KREBS BICARBONATE BUFFER (CO_2-N_2 MIXTURE)

	No. observations	Mean change (%)	S.E.M.	P
Cerebroside	5	− 9.58	±11.57	>0.4
Free cholesterol	6	+ 0.08	± 6.13	>0.9
Total cholesterol	6	+ 0.07	± 5.78	>0.9
Total phospholipid	6	−21.20	± 9.10	<0.1
Lecithin	5	−17.10	±15.10	>0.3
Sphingomyelin	5	−14.50	±11.50	>0.2
Cephalin	5	−27.60	± 8.12	<0.05

When Krebs bicarbonate buffer was used, with the exception of cephalin, there was still no significant change in lipid concentration. That there was no significant difference between the percentage change in the total phospholipid concentration when the brain was incubated in bicarbonate buffer with saline and when it was incubated in Krebs bicarbonate buffer confirms the work of Sperry (22) on rat brain homogenates.

A great decrease in total phospholipid, sphingomyelin, and lecithin was observed in brain slices accidentally contaminated with *Clostridium welchii*. These results are interesting, since this microorganism has been shown to hydrolyze lecithin and sphingomyelin (MacFarlane and Knight (13), MacFarlane (12)). Other contaminating organisms did not produce this change. All samples that gave a positive culture were discarded.

There was a significant decrease in total phospholipid, sphingomyelin, and cephalin after incubation for seven days in Krebs buffer in carbon dioxide – oxygen mixture, whereas only total phospholipid and cephalin were decreased significantly when the oxygen of the gas mixture was replaced by the nitrogen

(Table II). However, when the means of the values obtained for each lipid in carbon dioxide and nitrogen were compared with those obtained for the corresponding lipid in carbon dioxide and oxygen, no significant difference in the results was found. The drawing of any conclusion concerning a difference in behavior between slices incubated in oxygen and those incubated in nitrogen is, therefore, questionable.

When autolysis in carbon dioxide and nitrogen was prolonged to 14 days, there was a significant decrease in concentration of total phospholipid, sphingomyelin, and cephalin with no significant change in the concentration of lecithin (Table III). The concentration of cerebroside and cholesterol did not change significantly even after 14 days.

In Fig. 1 is presented the percentage change in the "essential lipids" i.e. cerebroside, total cholesterol, and total phospholipid. Each point is the mean percentage change of all samples incubated for similar periods of time
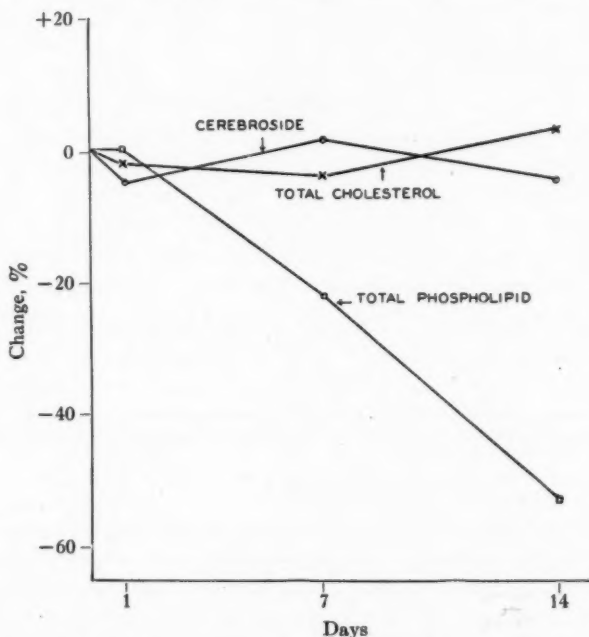
FIG. 1. *The percentage change in 'essential lipids' i.e. cerebroside, total cholesterol, and total phospholipid after incubation in bicarbonate buffer at 37° C. Each point represents the mean of 10 observations except of those for 14 days, which represent the mean of six observations.*

regardless of buffer or gas mixture. It can be seen that, whereas there was a great decrease in total phospholipid, there was no change in cerebroside or total cholesterol. Fig. 2 shows the percentage change of the individual

phospholipids.   There was a great change in the concentration of sphingo-
myelin and cephalin and no significant change in the concentration of lecithin.

In sciatic nerve, incubated for eight days in Krebs bicarbonate buffer,
there was a significant decrease in cephalin and a decrease of borderline
significance in total phospholipid (Table IV).   There was no significant
change in the concentration of cerebroside, free or total cholesterol, sphingo-
myelin, or lecithin.
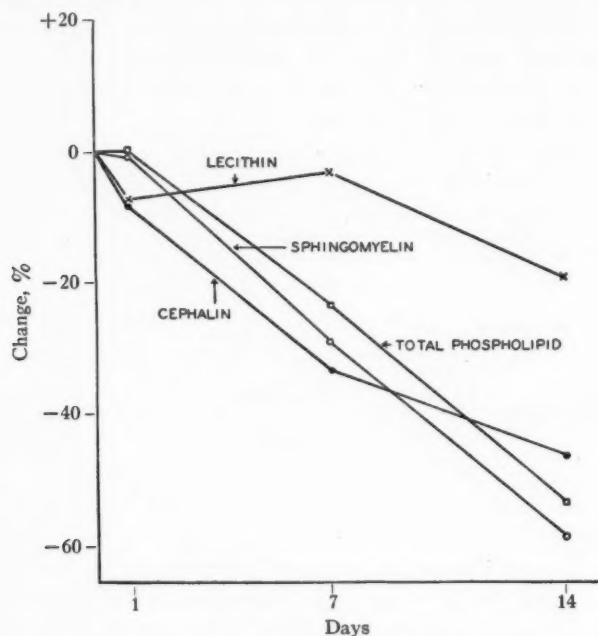
FIG. 2.   *The percentage change in phospholipids after incubation in bicarbonate buffer
at 37° C.   Each point represents the mean of 10 observations except those for 14 days, which
represent the mean of six observations.*

Discussion

When a peripheral nerve undergoes Wallerian degeneration there is little
change in the concentration of cerebroside, free cholesterol, or sphingomyelin,
the substances that we have previously suggested are the principal lipids of
the myelin sheath of nerve (6, 7, 9), or in the concentration of cephalin, during
the first eight days after nerve section (8).   When the nerve had autolyzed
for eight days *in vitro*, there was a significant decrease in cephalin, a decrease
of borderline significance in total phospholipid, with no significant change in
any of the other lipids.   Thus it appears that *in vitro* the nerve degenerated
more rapidly than *in vivo*.   In this connection it is interesting to recall the
observation of Cajal (2) that the histological changes characteristic of
Wallerian degeneration proceed more rapidly *in vitro* than *in vivo*.

A comparison between the *in vitro* autolysis of brain and the *in vivo* degeneration of nerve, already described (Johnson *et al.* (8)), is of interest. At the end of seven days, the changes in total phospholipid, sphingomyelin, and cephalin in brain were considerably greater than those observed for nerve at the end of eight days.   At the end of 14 days, the percentage change in phospholipid was similar.   In both cases there was a substantial fall in the concentration of cephalin and sphingomyelin and little change in lecithin. The results for the other lipids were, however, quite different.   In nerve degenerating *in vivo*, there was a definite decrease in the concentration of cerebroside and total cholesterol with a great increase in ester cholesterol. It thus appears that the enzyme systems concerned with the degeneration of nerve *in vivo* differ from those concerned with the *in vitro* autolysis of brain tissue.   This is perhaps hardly surprising, since, *in vivo*, macrophages appear along the course of the degenerating nerve and contribute their enzymes to the degradation mechanism.

It will be noted that there was little change in the concentration of lecithin in either autolyzing brain tissue or in nerve degenerating *in vivo*.   Lecithin was determined as the choline liberated from the mixed phospholipid extract after hydrolysis in N potassium hydroxide for 18 hr. at 37° C.   It is possible that the autolyzed sample contained unknown degradation products of either lecithin or sphingomyelin that would yield choline on hydrolysis under these conditions.   It is unlikely that the lecithin figures are unduly high for this reason, since known intermediates, such as α-glycerylphosphorylcholine studied by Schmidt, Hershman, and Thannhauser (20), are petroleum ether insoluble and would not be found in the phospholipid extracts.

Much of the early work on brain autolysis was concerned with the degradation of proteins rather than of lipids.   When the importance of the brain lipids became generally appreciated, reports appeared on the change in lipid content of brain during autolysis.   Page (18) advanced a hypothetical scheme for the biochemical changes in lipids during demyelination.   This scheme was based, in part, on the evidence of Jungmann and Kimmelstiel (10) and of Backlin (1).   The former workers found that cerebroside decreased and inorganic phosphorus increased when rabbit whole brain stood in either oxygen or nitrogen.   Backlin (1) described a fall in free cholesterol and cerebroside and a possible increase in phospholipid when a rabbit whole brain stood for 24 hr.   However, his figures are the results of only one experiment and are not significant.   Sperry, Brand, and Copenhaver (23) found little change in the concentration of total cholesterol and total phospholipid in one half of a rat's brain, incubated in 0.9% sodium chloride for one day. Under the conditions of our study, there was no change in cerebroside or total cholesterol of cat brain slices even after 14 days.   Although there was no change in total phospholipid after 24 hr., the decrease following longer

periods of incubation has been confirmed. It should be pointed out that many of the above workers did not mention a buffer system and it is likely that the hydrogen ion concentration was not controlled.

Simon (21) found that inorganic phosphorus, which appeared during brain autolysis, was derived from alcohol–ether soluble material, presumably phospholipid, a result confirmed by Stamm (24). Coriat (3) reported that choline was liberated when minced brain was autolyzed for 72 hr. in the presence of chloroform. The possibility that autolyzing brain tissue contains a lecithinase is suggested by the work of King (11), who found that dispersions of rabbit and chicken brain liberated small and variable quantities of free and acid soluble phosphorus from a lecithin emulsion. With homogenates the hydrolysis of phospholipids is apparently much faster. This is shown by the work of Fries *et al.* (5) and Sperry (22) who demonstrated a constant but small decrease in total phospholipid concentration when homogenates of brain tissue were incubated for only four hours.

A possible interpretation of these results that must be considered is that the physical properties of the phospholipids are altered in such a way that they are no longer extracted by the procedures employed. This problem was fully investigated by Sperry (22), who used a number of different extraction procedures and obtained similar results. His results, and our finding that cholesterol and the relatively insoluble cerebroside were completely extracted after incubation, would indicate that hydrolysis of phospholipid does occur.

This work suggests that in tissue of the central nervous system, there is a phospholipid-splitting mechanism, possibly enzymatic in nature. It has not been possible to demonstrate the degradation of either cerebroside or cholesterol.

References

1. BACKLIN, E. Beiträge zur Quantitativen Kenntnis der Gehirnlipoide. Almqvist, Uppsala. 1930.
2. CAJAL, S. Y. Degeneration and regeneration of the nervous system. Oxford University Press, London. 1928.
3. CORIAT, I. H. Am. J. Physiol. 12 : 353. 1904.
4. FALK, F. Biochem. Z. 13 : 153. 1908.
5. FRIES, B. A., SCHACHNER, H., and CHAIKOFF, I. J. Biol. Chem. 144 : 59. 1942.
6. JOHNSON, A. C., McNABB, A. R., and ROSSITER, R. J. Biochem. J. 43 : 573. 1948.
7. JOHNSON, A. C., McNABB, A. R., and ROSSITER, R. J. Biochem. J. 43 : 578. 1948.
8. JOHNSON, A. C., McNABB, A. R., and ROSSITER, R. J. Rev. can. biol. In press. 1949.
9. JOHNSON, A. C., McNABB, A. R., and ROSSITER, R. J. Biochem. J. To be published. 1949.
10. JUNGMANN, H. and KIMMELSTIEL, P. Biochem. Z. 212 : 347. 1929.
11. KING, E. Biochem. J. 25 : 799. 1931.
12. MACFARLANE, M. G. Biochem. J. 42 : 587. 1948.
13. MACFARLANE, M. G. and KNIGHT, B. C. J. G. Biochem. J. 35 : 885. 1941.
14. MAY, M. R. M. Bull. soc. chim. biol. 12 : 934. 1930.
15. MONCKEBERG, G. and BETHE, A. Arch. mikroskop. Anat. Entwicklungsmech. 54 : 135. 1899.

16. Mott, F. W. and Halliburton, W. D.   Trans. Roy. Soc. (London), B, 194 : 437.   1901.
17. Nageotte, J.   Compt. rend. soc. biol. 68 : 463.   1910.
18. Page, I. H.   Chemistry of the brain.   Charles C. Thomas. Publisher, Springfield & Baltimore.   1937.
19. Randall, L. O.   J. Biol. Chem. 125 : 723.   1938.
20. Schmidt, G., Hershman, B., and Thannhauser, S. J.   J. Biol. Chem. 161 : 523.   1945.
21. Simon, F.   Z. physiol. Chem. 72 : 463.   1911.
22. Sperry, W. M.   J. Biol. Chem. 170 : 675.   1947.
23. Sperry, W. M., Brand, F. C., and Copenhaver, W. M.   J. Biol. Chem. 144 : 297. 1942.
24. Stamm, W.   Arch. exptl. Path. Pharmakol. 111 : 133.   1926.

SOME FACTORS AFFECTING THE AUSCULTATORY MEASURE-MENT OF ARTERIAL BLOOD PRESSURES[1]

By A. E. Thomson and J. Doupe

Abstract

Auscultatory blood pressure measurements have been compared to intra-arterial lateral and end pressures. It was found that auscultatory measurements, which are dependent upon the penetration of pulse waves through a compressed segment of artery, were influenced by various factors. When auscultatory measurements approximated or exceeded intra-arterial pressures, broad pulses were found; when auscultatory measurements were below intra-arterial pressures, narrow pulses were found. By measuring tissue pressures under a cuff it was shown that cuffs subtended only a relatively short narrow band of equal pressure into the tissues. Hence narrow cuffs or, conversely, large arms that allowed only a fraction of the applied pressure to reach the artery caused high auscultatory measurements of both systolic and diastolic pressure. It was concluded that pulse contour and arm size were major causes of the auscultatory systolic errors while the diastolic errors were due to arm size plus unknown factors.

Introduction

It has been pointed out by Ragan and Bordley (2) that auscultatory measurements of blood pressure are frequently in error. In 40 patients the range of deviation of systolic measurements from lateral intra-arterial pressures was -20 to $+47$ mm. Hg, and for diastolic measurements the range was -12 to $+39$ mm. Hg. Their work and that of others (3, 4, 5) indicated that the auscultatory measurements were affected by the contour of the pulse, the girth of the arm, and the width of the blood pressure cuff. The present report is mainly concerned with the mechanism by which these factors produce their effects and is not primarily concerned with the extent of the error nor with the difficulties of interpretation caused by the variability of the pressure.

Method

The values obtained by the standard auscultatory procedure (7) have been compared to intra-arterial pressure measurements obtained by using a variable capacitator similar to that described by Lilly (1). A hollow 20 gauge needle inserted into the brachial artery transmitted the pressure to a phosphor-bronze diaphragm that formed one plate of a condenser in a radio frequency circuit. The position of the diaphragm controlled the frequency and this in turn determined the output of an amplifier that fed an oscillograph galvano-meter whose deflections were recorded on moving bromide paper. At approximately five-minute intervals during a recording, the manometric system was calibrated by the application of known pressures to the diaphragm. Measurements approximating lateral pressure were obtained by this method. To obtain end pressures the circulation was occluded by a cuff immediately distal to the needle. The frequency characteristics of the apparatus were

not accurately ascertained but the response time of the system, with attached 20 gauge needle, for a sudden fall in pressure from 200 to 0 mm. Hg, was less than 5 msec.   The other methods employed will be described with the results.

Subjects with appropriate arm sizes, who did not exhibit obvious cardio-vascular disorders, were selected, and all measurements were taken with the subjects at rest in the recumbent position.   In certain cases the circulation to the extremity was increased by general body heating and vigorous exercise of the arm muscles.   Arm circumference was measured at a point midway between the acromion and the olecranon.   In the subjects in whom the intra-arterial pressures of one arm were compared to the auscultatory measurements of the other arm it was required that the auscultatory measurements of the two arms did not differ by more than 5 mm. Hg.

Results

In common with those of other investigators the present results have shown certain discrepancies between auscultatory and intra-arterial pressure measurements.   These will be described first and related as far as possible to the pulse contour, then the influence of the width of the blood pressure cuff, and the girth and consistency of the arm will be considered and finally observations of the tissue pressure will be reported.

Auscultatory and Lateral Pressures

Simultaneous auscultatory and lateral pressure measurements were obtained three to five times in 13 experiments on 10 subjects.   As the relationship between the two measurements in each experiment was constant to within 5 mm. Hg, only their averages are recorded in Table I.   In these experiments

TABLE I

SIMULTANEOUS AUSCULTATORY AND INTRA-ARTERIAL PRESSURES

(Measured in mm. Hg)

Subject	Arm girth, cm.	Crest time,** msec.	Systolic			Diastolic		
			Ausc.	Lateral	Diff.	Ausc.	Lateral	Diff.
D.C.	25	120	147	157	− 10	79	62	+ 11
E.L.	23	130	120	122	− 2	70	65	+ 5
V.M.	26	160	131	145	− 14	70	70	+ 0
H.J.	23	170	125	126	− 1	80	52	+ 28
H.K.	23	200	102	105	− 3	68	56	+ 12
M.G.	21	200	151	148	+ 3	53	57	− 4
G.T.	26	200	165	162	+ 3	80	59	+ 21
R.F.	27	200	118	120	− 2	78	64	+ 14
P.B.	27	220	124	121	+ 3	79	65	+ 14
V.M.*	26	300	136	131	+ 5	78	56	+ 22
H.K.*	23	300	101	96	+ 5	70	58	+ 12
T.K.	30	330	124	116	+ 8	81	69	+ 12
T.K.*	30	400	122	112	+ 10	81	76	+ 5

* Subjects in whom blood flow was increased by heat and exercise.
** Measurement described in text.

auscultatory systolic measurements averaged 0.4 mm. Hg above lateral systolic pressure with a range of difference from minus 14 to plus 10 mm. Auscultatory diastolic measurements, using muffling of sounds as an index, averaged 12.4 mm. Hg above lateral diastolic pressure with a range of difference from minus 4 to plus 28 mm. This is in accord with the findings of other investigators (2, 4, 5). The discrepancy between diastolic measurements disappeared if the abrupt cessation of sounds was used as a criterion for the auscultatory measurement as reported by Steele (5), but ordinarily the transition was so gradual that this point could not be detected.

Contour of the Lateral Pressure Pulses

Information as to one of the causes of the observed discrepancies of the systolic measurements was obtained by analyzing the contour of the lateral pressure pulses in the individual cases. The shape of the pulses differed from individual to individual and in the same individual with changes in circulatory state. To obtain an index of their relative peakedness the average duration of three representative waves 15 mm. Hg below the systolic peak was determined. These measurements of "crest time" appear in Table I and the subjects have been arranged so that they progress from those with the most peaked to those with the most rounded pulses. It will be seen that the auscultatory systolic measurements were below lateral pressures when the pulse crest was narrow, approximately the same when the crest was intermediate in type, and above when the crest was broad. No such relationship could be demonstrated for the discrepancies in diastolic measurements. These observations are also in agreement with those reported by others (2, 4).

Difference Between End and Lateral Pressures

Despite the observed relationship of pulse contour to the difference between auscultatory and lateral systolic pressures it seemed unjustified to stress this relationship when theoretical considerations suggested that end and not lateral pressures should be used for the comparison. To test this possibility auscultatory measurements were made before and after end pressures had been produced by inflating a cuff on the forearm to beyond systolic pressure. This was done three times on four subjects both before and after heating and exercise. In no instance did inflation of the cuff produce a change greater than 4 mm. Hg in either the systolic or diastolic measurement. To show that obstruction of the flow did cause a significant change in pressure eight experiments were done on five subjects by inflating a cuff just distal to the site of arterial puncture. For comparison three measurements of pressure were made prior to the inflation of the cuff and three measurements after inflation in approximately the same phase of respiration. The averages of these observations are presented in Table II. In every instance a rise in systolic pressure occurred and, for the group, averaged 9 mm. Hg with a range of 3 to 17 mm. This rise in pressure was accompanied by a change in pulse contour and the extent of the rise was related to the degree of narrowing of the pulse as

TABLE II

END AND LATERAL INTRA-ARTERIAL PRESSURES

(Measured in mm. Hg)

Subject	End pressure		Lateral pressure		Difference (end minus lateral)		% narrow-ing of pulse**
	Sys.	Dias.	Sys.	Dias.	Sys.	Dias.	
E.L.	140	63	125	61	15	2	33
V.M.	166	68	149	63	17	5	32
H.K.*	109	61	98	59	11	2	33
T.K.*	121	81	113	79	8	2	30
T.K.	117	73	112	70	5	3	25
V.M.*	119	68	113	67	6	1	13
H.K.	116	59	113	56	3	3	10
M.G.	149	58	146	57	3	1	0

* Subjects in whom blood flow was increased by heat and exercise.
** Calculated from Crest Times in Tables I and III.

shown in Table II. As in the case of auscultatory diastolic measurements no significant change was produced in intra-arterial diastolic pressure by obstructing the flow. These observations agree in general with those of Robinow *et al.* (4).

Because systolic end pressures differed materially from lateral pressures and because auscultatory systolic measurements were not affected by obstruction of the blood flow, it was apparent that auscultatory measurements are made when end pressures exist and therefore should be compared to them.

Auscultatory Measurement and End Pressures

The comparison between systolic auscultatory and end pressures has been made in Table III utilizing data from Tables I and II. It will be seen, owing

TABLE III

DISCREPANCY BETWEEN AUSCULTATORY AND END SYSTOLIC PRESSURES

Subject	E.L.	V.M.	H.K.	M.G.	H.K.*	T.K.*	T.K.	V.M.*
End crest time (msec.)	80	100	180	200	200	230	250	260
Ausc. minus end (mm. Hg)	−17	−31	−6	0	−6	−2	−3	−1

* Subjects in whom blood flow was increased by heat and exercise.

to the alterations produced in the pulse wave contour and in the systolic pressure by obstructing the flow, that the discrepancies between auscultatory and intra-arterial pressures have been altered. The net effect of changing

the standard of comparison from lateral to end pressures has been to accentuate the differences associated with narrow pulses and to minimize those associated with broad pulses.

Influence of Cuff Width and Arm Girth

To show the influence of cuff width and arm girth on auscultatory measurements, a group of subjects was chosen in which there was a much wider variation in regard to arm circumference than in the group just considered. On 18 subjects, whose arm circumference ranged from 20 to 38 cm., three auscultatory measurements of systolic and diastolic pressure were made with each of four cuffs, 8, 12, 16, and 20 cm. in width. The group was divided into three equal classes, those with arm circumferences from 20 to 26 cm., from 26 to 32 cm., and over 32 cm. The mean values of each class for the four widths of cuff are plotted in Fig. 1, which shows that the highest measure-
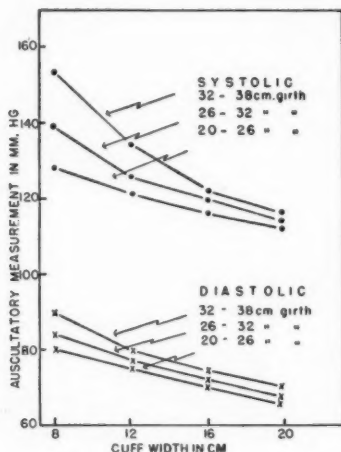
Fig. 1. *Effect of cuff width on auscultatory measurements in subjects with different arm girths.*

ments of both systolic and diastolic pressure were obtained using the 8 cm. cuff and the greater the girth of arm the greater the effect of changing the width of the cuff, the initial slope of the curves being steepest in the class with the greatest arm girths.

As the above results indicated the importance of arm girth, an attempt was made to ascertain the relationship of measured girth to effective girth, i.e., the girth after inflation of the cuff. To determine this, using a soft tissue technique, X-ray films were taken before and after inflation of a standard blood pressure cuff to systolic pressure. The measured girths of three subjects whose soft tissues ranged from firm to flabby were 29, 30, and 34 cm. Girths calculated from X-ray films before inflation were 29, 30, and 35 cm. and after

inflation 27, 26, and 30 cm. Clearly effective girth differed from measured girth and this difference appeared to depend upon the consistency of the soft tissues.

Distribution of Pressure in the Tissues Under a Cuff

The pressure in the tissues under a blood pressure cuff was measured using a modification of the apparatus described by Wells *et al.* (6) in which the water in the measuring column was replaced by mercury.   Measurements were made using four or more cuff pressures between 20 and 200 mm. Hg at four points under two cuffs of different widths.   Those for the 12 cm. cuff are plotted in Fig. 2 and show that for each point in the tissues the ratio of tissue pressure
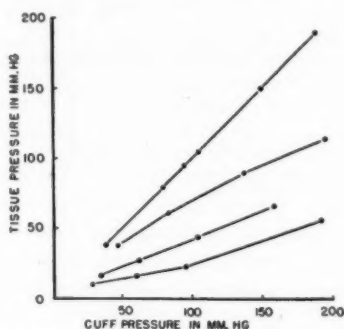
FIG. 2.   *Effect of varying the cuff pressure on the pressure at four positions in the tissues.*

to applied pressure was constant.   The constancy of this ratio indicated that the spatial distribution of the relative pressure beneath a cuff was independent of the level of the cuff pressure.

The distribution of pressure was ascertained on one individual by measuring the tissue pressure at 29 different points under a 12 cm. cuff, nine under an 8 cm. cuff, and 26 under a 4 cm. cuff.   The position of these points was estimated by the depth of insertion and the angle of the needle and was probably accurate to within 0.5 cm. in any direction.   All the measurements were converted into percentage values of the cuff pressure and were located in the diagram shown in Fig. 3, keeping constant their relationship to the nearest edge of the cuff and their depth in the tissues.   Because the discrepancies between the measurements made with different cuffs were no greater than those between the measurements made with the same cuff, it was concluded that the pattern of the pressure distribution was similar for the various cuffs. The total number of observations was insufficient to enable exact isobaric lines to be drawn, but a general pattern was obvious.   The highest relative pressures were found near the center of the cuff, and the zone occupied by these values, although fairly extensive immediately beneath the cuff, became less so at greater depths in the tissues.   With the 4 cm. cuff the 100% zone was not detected.   Lowest relative pressures were found towards the edge of the cuff and the area occupied by these values was definitely greater at increasing

depths in the tissues.   The zone of intermediate values appeared to broaden
at greater depths in the tissues at the expense of the high pressure zone.
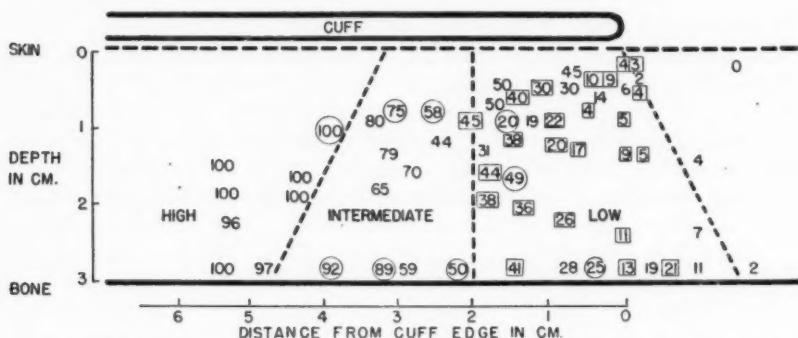
FIG. 3.   Diagrammatic longitudinal section of arm showing distribution of pressure in the
tissues under a cuff.   Pressures are expressed as a percentage of cuff pressures.   Those within
squares and circles and the others were obtained with 4, 8, and 12 cm. cuffs, respectively.
Borders of zones are indicated by dotted lines.

The application of the above results to the present problem was shown by
making auscultatory measurements on the same patient with different
widths of cuffs.   These are shown in Fig. 4 together with estimates of the
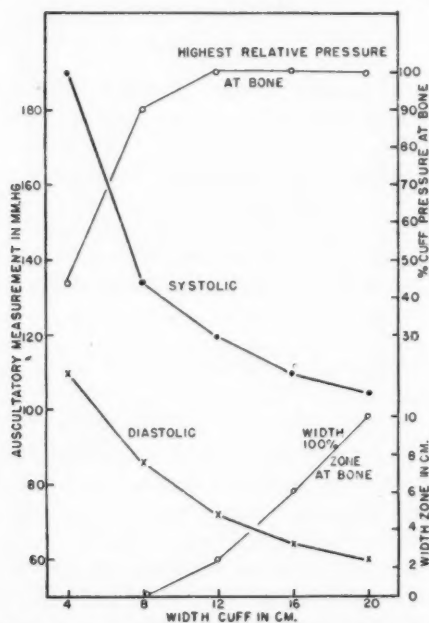
FIG. 4.   Effect of cuff width on auscultatory measurements compared to estimates of
relative tissue pressure at the level of bone.

relative pressure at the level of bone taken from Fig. 3. The steep parts of the systolic and diastolic curves appear to correspond to the transition from a cuff that did not project a high relative pressure zone as far as the bone to one that did. The less steep parts of the curves correspond to increases in width of the high pressure zone produced by changing to still wider cuffs.

Discussion and Summary

The present results indicate some of the reasons why auscultatory measurements are inaccurate. It was first shown that auscultatory systolic measurements were influenced by the shape of the pulse wave even after taking into consideration the phenomena associated with the conversion of lateral to end pressures. The explanation of this relationship seems obvious. The production of audible sounds depends upon the penetration of the peaks of the pulse waves through the segment of artery compressed by the cuff. A certain amount of energy is necessary to overcome the pressure exerted by the cuff. A pulse that is broad and rounded will have considerably more energy available at its peak to penetrate the compressed segment than will a narrow pulse. Thus when auscultatory systolic measurements approximated or exceeded intra-arterial pressures broad pulses were found; when auscultatory measurements were below intra-arterial pressures, narrow pulses were found. This theory also affords an explanation for the lack of relationship between the diastolic errors and the shape of the pulse waves.

It was then shown in a series of experiments, which were essentially a repetition of those of von Recklinghausen (3), that the use of narrow cuffs gave high systolic and diastolic auscultatory readings. This would be expected because it may be presumed that a narrow segment of compression will offer less resistance to a pulse wave than will a wide one, necessitating a higher compression pressure on a narrow segment to produce an equivalent resistance. However, the results also showed that this phenomenon was not similar in all ranges of arm girth. In the larger arms, narrow cuffs required a much higher pressure to obstruct the pulse than was expected. The observations of the tissue pressure under various cuffs showing that only a fraction of the applied pressure was being transmitted to the artery provided an explanation for these findings.

It was also shown that the consistency of the tissues affected the distortion of the arm caused by inflating a blood pressure cuff. This would make it difficult to deduce the effective girth from measurements of the uncompressed arm. It would appear likely, although the point was not investigated, that the consistency would also affect the distribution of pressure in the tissues.

The application of these results to clinical work merits attention. At the present time it may be justifiable on practical grounds to ignore the effect of pulse contour on systolic measurements because the significance of the more transient peaks of pressure is not known. The auscultatory method would even have an advantage, if it could be shown that such transient peaks

were so low in energy as not to materially affect either the walls of the blood vessels or the rate of flow. However, it is not justifiable to disregard the effect of arm girth particularly as this also modifies the diastolic measurement. Finally it should be stressed that the cause for many of the diastolic discrepancies has evaded analysis.

Acknowledgments

References

1. LILLY, J. C., LEGALLAIS, J., and CHERRY, R.   J. Applied Phys. 18 : 613.   1947.
2. RAGAN, C. and BORDLEY, J.   Bull. Johns Hopkins Hosp. 69 : 505.   1941.
3. RECKLINGHAUSEN, H. VON.   Arch. exptl. Path. Pharmakol. 46 : 78.   1901.
4. ROBINOW, M., HAMILTON, W. F., WOODBURY, R. A., and VOLPITTO, P. P. Am. J. Diseases Children, 58 : 102.   1939.
5. STEELE, J. M.   J. Mt. Sinai Hosp. N.Y. 8 : 1042.   1942.
6. WELLS, H. S., JOUMANS, J. B., and MILLER, D. G.   J. Clin. Invest. 17 : 489.   1938.
7. WRIGHT, I. S. and CAMPBELL, M.   Chairmen, the Committee for the Standardization of Blood Pressure Readings of the American Heart Association and the Committee for the Standardization of Blood Pressure Readings of the Cardiac Society of Great Britain and Ireland, respectively.   J. Am. Med. Assoc. 113 : 294.   1939.

EFFECT OF AMYTAL ON SKELETAL MUSCLE[1]

By Bernard E. Riedel and Mervyn J. Huston

Abstract

Sodium amytal, 190 mgm. per kgm., injected intraperitoneally into rats caused a transient increase in response of normal and denervated striated muscle to electrical stimulus. That this effect is not due to changes in pH nor to changes in ionic balance has been shown by pH tests on rat blood and by intraperitoneal administration of solutions of sodium hydroxide. It is believed that the increased response is due, at least in part, to a direct action on the muscle.

Introduction

Gross and Cullen (4) have shown that sodium pentothal in very high blood concentration depresses the response of striated muscle to intra-arterial injections of acetylcholine. A depressant effect of pentobarbital on the knee jerk and flexion reflex was reported by Van Harreveld (18). Huston, Martin, and Dille (11) demonstrated that pentobarbital and seconal depress the somatic neuromyal junction at concentrations 10 to 20 times the estimated effective concentrations at other synapses. At this concentration, however, an initial stimulant effect on the muscle was noted. It was thought of interest to examine further the action of barbiturates on the somatic system. This paper will deal with the results obtained with sodium amytal on the rat.

Method

The spinal cord of the rat was sectioned at the upper lumbar region under ether anesthesia and the rat left for 24 hr. to recover from the effects of the anesthetic. The animal was then tied down on its back on a small table and one hind leg was securely clamped at the knee and ankle. The calcaneous tendon was freed from the skin and fascia and severed at its attachment to the calcaneous bone. Recording was by means of an isometric spring attached to the calcaneous tendon.

The sciatic nerve was cut and the peripheral stump clamped in a shielded electrode. When denervated preparations were used, stimulation was through leads stitched in at each end of the gastrocnemius muscle. Stimulus was provided by an electronic stimulator at 10-sec. intervals. A maximal stimulus was used throughout.

A normal level of response to stimulation was obtained for approximately 90 min. before administration of the drug. The measurement of response

was made from the resting level of the muscle and was calculated as force of contraction in grams on a calibrated isometric spring. Experimental changes were reported as a percentage of the contraction tension before injection.

Results

Since the response of the normal muscle was found to decrease slowly, it was necessary to run a series of control animals to establish a criterion for the experimental results. Table I presents the results obtained with 20 normal rats. It will be noted that the mean shows a slow decline in response.

TABLE I

RESPONSE OF NORMAL GASTROCNEMIUS MUSCLE OF THE RAT TO
ELECTRICAL STIMULUS AT 10 SEC. INTERVALS

Figures represent percentage of initial contraction tension

Time in minutes								
3	6	9	12	15	30	45	60	72
100.0	100.0	100.0	100.0	100.0	101.4	101.4	101.4	103.6
97.2	97.2	97.2	97.2	94.5	92.6	—	—	81.9
100.0	100.0	100.0	100.0	98.2	95.3	86.7	80.4	80.4
100.0	95.1	95.1	95.1	91.6	91.6	89.6	84.5	84.5
100.0	100.0	97.3	97.3	96.2	96.2	92.4	88.8	87.0
100.0	100.0	101.0	101.0	101.0	101.0	101.0	101.0	101.0
100.0	100.0	99.0	99.0	99.0	98.2	99.2	99.2	99.2
100.0	100.0	100.0	100.0	100.0	95.4	90.3	88.9	84.2
99.0	99.0	99.0	99.0	99.0	99.0	97.6	96.8	94.4
102.1	102.1	99.4	99.4	99.4	98.6	97.3	97.3	97.3
97.3	97.3	97.3	97.3	93.4	97.3	94.5	92.3	89.6
102.4	100.0	102.4	108.8	108.8	106.4	102.4	102.4	102.4
98.0	93.5	89.0	87.5	85.0	82.0	74.5	76.0	71.5
99.0	104.9	95.1	90.7	88.3	81.9	81.9	84.4	77.6
94.5	94.5	88.3	91.0	88.3	89.6	100.0	94.5	88.3
101.0	104.6	107.2	103.1	101.0	107.2	107.2	108.2	103.1
98.7	98.7	101.7	97.8	93.4	98.7	98.3	95.2	93.9
100.0	101.9	100.0	100.0	96.6	98.1	91.2	91.9	91.9
100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
98.6	98.6	101.0	101.0	101.0	94.8	91.0	91.0	91.0
Mean 99.4	99.4	98.5	98.3	96.7	96.3	94.6	93.4	91.1
S.d.m. 0.39	0.65	0.96	1.02	1.22	1.44	1.79	1.86	2.04

The results obtained with 20 experiments when 190 mgm. per kgm. of sodium amytal was injected intraperitoneally are recorded in Table II. In every case there was an increased response followed by a progressive decrease. In 14 of 20 cases a maximal had been reached within 12 min. after the injection. The maximal mean increase was 13.7%. The highest single increase was 69.2%, which was not reached until 30 min. after the administration of the drug (No. V, Table II). As the dosage level used was very high for rats (14, 15, 16) a number of the animals died within a short time. No readings were taken after respiration ceased.

TABLE II

EFFECT OF SODIUM AMYTAL, 190 MGM. PER KGM., ON THE RESPONSE OF THE NORMAL GASTROCNEMIUS MUSCLE OF THE RAT

Figures represent percentage of initial contraction tension

				Time in minutes					
3	6	9	12	15	30	45	60	72
110.0	112.0	110.0	95.0	79.0	—	—	—	—
102.2	111.2	114.6	116.8	119.1	125.8	119.1	114.6	112.4
100.7	102.8	102.8	105.6	101.4	106.3	106.3	106.9	104.9
103.6	105.4	103.6	103.6	100.0	73.2	66.1	65.2	60.7
133.8	153.8	161.5	166.2	161.5	169.2	156.9	143.1	130.8
104.8	109.7	112.7	106.1	95.8	86.1	61.8	71.5	77.6
103.6	108.8	103.6	107.3	106.6	91.2	92.7	92.7	89.1
109.6	112.3	116.4	119.2	112.3	—	—	—	—
100.0	106.0	97.3	96.7	96.0	—	—	—	—
108.6	113.3	113.3	115.2	113.3	—	—	—	—
118.1	137.3	137.3	137.3	112.1	100.0	96.4	—	—
102.8	107.4	110.2	114.8	112.0	112.0	112.0	114.8	120.4
100.0	113.0	113.0	121.0	123.0	124.0	—	—	—
106.0	115.9	121.9	115.9	112.2	106.1	102.4	—	—
104.8	114.3	117.1	112.4	107.6	—	—	—	—
100.0	106.4	106.4	112.8	93.6	76.9	69.2	74.4	74.4
102.8	102.8	107.6	107.6	107.6	—	—	—	—
102.0	120.4	108.2	100.0	87.8	67.4	51.0	32.7	32.7
104.5	104.5	110.7	114.3	119.6	119.6	107.1	89.3	58.9
100.7	102.6	103.7	105.9	108.1	99.3	—	—	—
Mean 105.9	112.9	113.6	113.7	108.4	104.1	95.1	90.5	86.2
S.d.m. 1.76	2.77	3.15	3.46	3.70	7.10	8.43	10.15	9.80

In Fig. 1 a comparison of the normal mean (Table I) and the experimental mean (Table II) is presented graphically. It is apparent that there is a significant initial increase in response in the experimental legs but that the subsequent depression is not significantly different from the control.
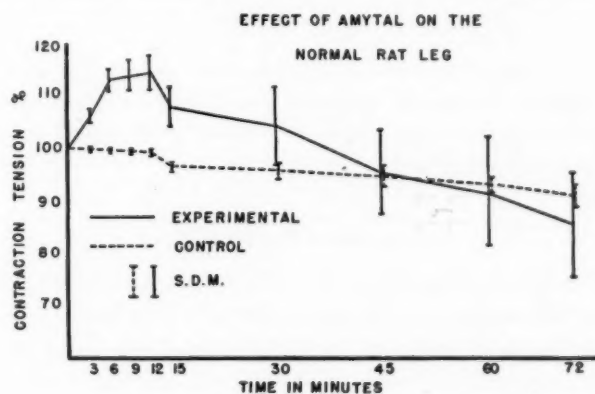
FIG. 1.

It was considered possible that the stimulant effect might be due to the alkalinity of the solution or to the sodium ion.   Comparisons were made by means of a glass electrode of the pH of blood from normal and injected rats. No significant difference was noted.   The buffers of the blood can apparently compensate for the amount of sodium amytal used.   To establish that pH and ionic factors are not involved in the increased response, a series of experiments was run in which sodium hydroxide solution was injected intraperitoneally.   The dose of sodium hydroxide used contained a weight of sodium equivalent to the weight of sodium in an experimental dose of sodium amytal.   The pH of the sodium hydroxide solution was 10.8.   This is higher than the pH of sodium amytal solution, which was 9.9.   Table III presents

TABLE III

EFFECT OF SODIUM HYDROXIDE ON THE RESPONSE OF THE NORMAL
GASTROCNEMIUS MUSCLE OF THE RAT

(Sodium equivalent to that of sodium amytal 190 mgm. per kgm.)

Figures represent percentage of initial contraction tension

				Time in minutes				
3	6	9	12	15	30	45	60	72
97.4	97.4	97.4	97.4	99.1	99.1	99.1	99.1	99.1
100.0	97.3	97.3	100.0	100.0	118.3	118.3	118.3	118.3
100.0	100.0	100.0	100.0	103.0	90.0	78.0	75.0	75.0
100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	97.5
100.0	97.6	97.6	97.6	97.6	96.0	83.0	68.5	66.6
100.0	100.0	96.3	93.9	93.0	90.7	86.5	82.8	76.2
100.0	100.0	100.0	98.4	96.0	94.4	89.4	89.4	89.4
Mean 99.6	98.9	98.3	98.2	98.4	98.4	93.5	90.4	88.9
S.d.m. 0.37	0.48	0.55	0.83	1.22	3.61	5.11	6.40	6.70

the results obtained with seven such experiments.   It is apparent from Fig. 2, where the mean of the normal legs is compared graphically to the mean of the legs treated with sodium hydroxide, that there is no significant difference. It is therefore believed that pH and ionic effects are not responsible for the stimulation.

Denervation Experiments

In an attempt to localize the stimulating action, we performed a series of experiments with sodium amytal upon denervated muscles.

The animals were prepared by aseptic section of the sciatic nerve in one thigh.   After a period of five days to allow for nerve degeneration, these limbs were studied by the method described above.

Since the decrease of the denervated muscle might be expected to differ from that of nondenervated muscle, a series was run as a control. The

FIG. 2.
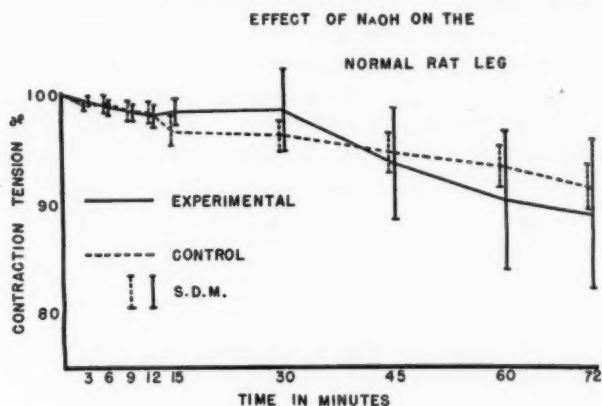
results with 12 such rats are tabulated in Table IV. It will be noted by comparison with Table I that the decrease with denervated muscle is slightly more rapid than with normal muscle.

TABLE IV

RESPONSE OF DENERVATED GASTROCNEMIUS MUSCLE OF THE RAT TO
ELECTRICAL STIMULUS AT 10 SEC. INTERVALS

Figures represent percentage of initial contraction tension

Time in minutes								
3	6	9	12	15	30	45	60	72
97.6	96.1	92.9	88.2	84.3	74.2	74.2	68.7	65.1
97.6	94.3	94.3	93.2	91.0	85.4	82.0	78.6	78.6
98.1	93.5	91.7	91.7	91.7	77.4	68.8	63.3	57.8
100.9	100.9	100.9	96.0	96.0	100.9	93.1	99.0	81.3
96.3	94.4	94.4	94.4	91.6	87.9	87.0	83.3	83.3
100.0	100.0	100.0	100.0	100.0	98.4	96.9	94.5	94.5
100.0	95.0	95.0	92.6	90.1	89.3	89.3	86.8	86.8
100.0	100.0	97.7	97.7	97.7	97.7	95.4	96.9	96.9
100.0	100.0	100.0	100.0	100.0	97.5	97.5	97.5	97.5
100.0	100.0	100.0	100.0	99.1	99.1	98.3	96.0	96.0
97.5	93.0	88.6	87.1	84.6	81.6	74.1	75.6	71.1
99.4	97.3	94.5	96.2	93.4	87.9	83.1	83.1	83.1
Mean 99.0	97.0	95.8	94.8	93.3	89.8	86.6	85.3	82.7
S.d.m. 0.41	0.86	1.11	1.25	1.57	2.57	2.91	3.42	3.69

Table V presents the results obtained when 190 mgm. per kgm. of sodium amytal is injected into rats with denervated legs. The pattern of events is similar to that obtained with normal rats. There is an increase of response

TABLE V

EFFECT OF SODIUM AMYTAL, 190 MGM. PER KGM., ON THE RESPONSE OF THE
DENERVATED GASTROCNEMIUS MUSCLE OF THE RAT

Figures represent percentage of initial contraction tension

				Time in minutes				
3	6	9	12	15	30	45	60	72
105.0	111.7	106.7	105.8	103.3	84.2	70.8	—	—
100.9	108.2	102.7	98.2	93.6	75.5	80.0	81.8	—
103.4	105.7	105.7	109.1	109.1	100.0	71.6	—	—
103.3	105.6	—	108.9	108.9	103.3	106.7	103.3	103.3
91.6	100.0	105.9	105.9	110.1	107.6	109.2	103.4	100.8
102.4	109.4	109.4	103.5	97.7	74.2	82.4	88.2	88.2
101.2	109.4	109.4	108.2	105.9	97.7	95.3	91.8	88.2
100.0	102.4	105.9	108.2	108.2	90.6	—	—	—
101.8	109.2	109.2	109.2	108.3	103.7	—	—	—
100.0	103.8	102.3	100.0	98.5	82.7	—	—	—
110.0	107.1	102.9	102.9	100.0	58.3	—	—	—
103.5	101.2	97.7	90.7	90.7	59.3	—	—	—
100.0	99.2	92.4	87.0	80.9	60.3	60.3	58.8	58.8
102.1	104.5	104.5	101.7	98.8	86.4	82.3	75.7	71.6
107.5	105.4	102.7	101.6	123.5	—	—	—	—
100.0	99.0	95.0	92.5	88.0	75.0	68.5	64.0	59.5
105.4	101.4	97.3	84.4	78.2	—	—	—	—
100.0	102.6	105.3	100.0	100.0	72.8	—	—	—
101.9	104.7	104.7	99.5	—	—	—	—	—
103.3	101.1	90.1	78.0	73.6	29.7	17.6	16.5	16.5
Mean 102.2	104.6	102.6	99.7	98.8	80.1	76.8	75.9	73.4
S.d.m. 0.80	0.82	1.30	1.90	2.84	4.26	7.60	9.07	13.75

that reaches a mean peak of 4.6% in six minutes, followed by a decrease.
Fig. 3 compares the contraction tension of the denervated experimental legs
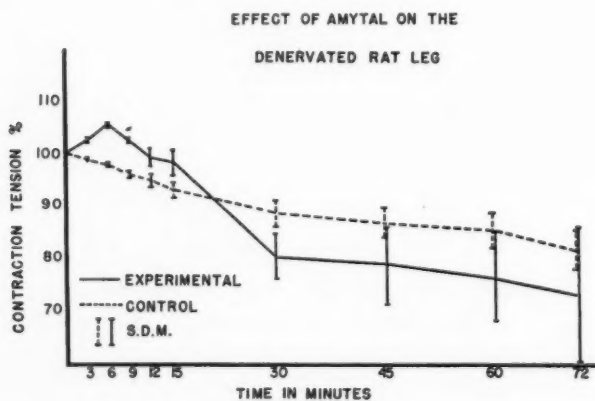with those of the denervated control group.

FIG. 3.

Although the increase with the denervated legs is less than with the normal legs this may be due to the more rapid fatigue of the denervated muscle. Table VI presents a comparison of the effects of amytal on normal and

TABLE VI

COMPARISON OF EFFECT OF SODIUM AMYTAL, 190 MGM. PER KGM., ON NORMAL AND
DENERVATED GASTROCNEMIUS MUSCLE OF THE RAT

Figures represent experimental mean as percentage of corresponding control mean

	Time in minutes								
	3	6	9	12	15	30	45	60	72
Normal muscle	106.6	113.6	115.3	115.7	112.1	108.1	100.5	96.9	94.6
Denervated muscle	103.2	107.8	107.1	105.2	105.9	89.2	88.7	88.9	88.7

denervated muscle when calculation is based on the control curves.   There is a peak increase of 15.7% at 12 min. with normal muscle as compared with a peak of 7.8% at six minutes with denervated muscle.   Fig. 4 compares the

COMPARISON OF EFFECT OF AMYTAL ON NORMAL AND DENERVATED RAT LEGS
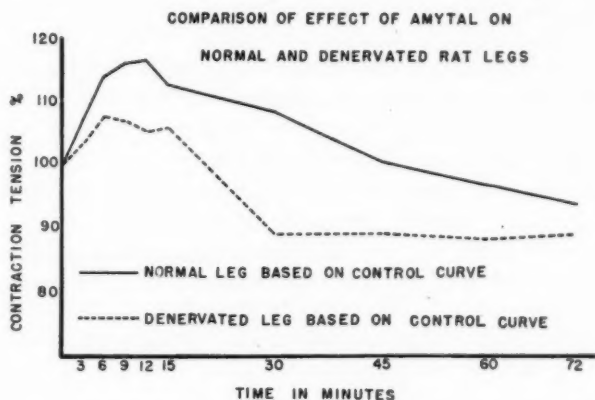
FIG. 4.

effect on normal and denervated muscle graphically.   It would appear that at least part of the effect of amytal is attributable to a direct action on muscle. However, the increased response with denervated muscle was only about half that with normal muscle.   This does not necessarily mean that only half the effect on normal muscle is due to a direct action.   Denervation is known to cause profound changes in muscle (13, 17) so that the lower response of denervated muscle may be due to the fact that the denervated muscle is less robust and therefore less able to respond to a stimulant.

Discussion

Huston, Martin, and Dille (11) reported a stimulant effect of pentobarbital and seconal on striated dog muscle, which preceded a depressant effect shown to be on the neuromyal junction.   They attributed the stimulant effect to a direct action on the muscle.

Our experiments with sodium amytal therefore agree with the increased response they obtained with pentobarbital and seconal.   We were unable to demonstrate any significant depressant action on the neuromyal junction. This is probably due to the fact that we were unable to reach a sufficiently high concentration in the intact animal without causing death by central depression.   Huston et al. obtained concentrations in an isolated perfused leg that were much higher.   The degree of increase noted by us is much less than that reported by Huston et al.   This may be due to our lower concentration but might also be due to a quantitative difference between amytal, pentobarbital, and seconal.   It is known that barbiturates differ quantitatively in their effects on the same part of the nervous system.   Such differences on the central nervous system are well known but also have been reported at peripheral points (9).   Similar differences might exist in effect on muscle.

The depressant effect that sodium pentothal has on the response of striated muscle to intra-arterial injection of acetylcholine (4), and the depressant effect that pentobarbital and seconal have on the neuromyal junction (11), may be related to the decrease in response of the heart to vagus stimulation under the influence of high dosages of pentobarbital, amytal, and evipal (9), and to the depression of the motility of the gastrointestinal tract by barbiturates (2, 6, 7, 8).   There is, however, considerable difference in the sensitivity of different parts of the nervous system to barbiturates (11).

On the other hand a stimulant action of barbiturates on smooth and cardiac muscle has not been reported.   This may be due to a difference in response of different types of muscle or it may be that slight or transient effects have been overlooked.   Gruber et al. (10) in experiments on excised smooth muscle noted a stimulating action with barbiturates but attributed this to the alkalinity of the solution since the effect was not apparent after control of the pH.

A slight decrease in muscle tone was noted after injection of amytal.   As calculations are made from the resting or tone level this contributes to the figures recorded.   The decrease in tone was slightly more marked in the case of denervated muscle.   A similar depression of the tone of smooth muscle by barbiturates has been noted.   Gruber and his co-workers have reported a marked decrease in tonus of longitudinal muscle of the intestine (5); a decrease in tonus of stomach muscle (8); and a decrease in general tonus of intact intestine in the unanesthetized dog (2, 6).

It is interesting to note in the case of striated muscle that a decreased tone occurs at the same time as an increased degree of contraction to stimulus.

That the two phenomena are not intimately linked is seen from the fact that the tonus level remains essentially the same after the brief period of stimulation is over.

Denervation of muscle results in a sensitization to acetylcholine (1, 12, 17), which is believed by Cannon and Rosenbleuth (3) to be one example of the general condition of the muscle. Sensitization to barbiturates has not been demonstrated. We believe our experiments to be free from the effect of sensitization for the reasons previously stated (11) and because the stimulant effect of amytal was less with denervated muscle than with normal muscle. Our experiments with amytal would therefore lend support to the contention that sensitization was not of significance in the experiments with pentobarbital and seconal (11).

References

1. BROWN, G. L.   J. Physiol. (London), 89 : 438.   1937.
2. BURSTEIN, C. L.   Proc. Soc. Exptl. Biol. Med. 40 : 122.   1939.
3. CANNON, W. B. and ROSENBLUETH, A.   Am. J. Physiol. 116 : 408.   1936.
4. GROSS, E. G. and CULLEN, S. C.   J. Pharmacol. Exptl. Therap. 78 : 358.   1943.
5. GRUBER, C. M.   J. Pharmacol. Exptl. Therap. 30 : 149.   1926-1927.
6. GRUBER, C. M.   J. Pharmacol. Exptl. Therap. 56 : 432.   1936.
7. GRUBER, C. M., CRAWFORD, W. M., GREEN, W. W., and DRAYER, C. S.   J. Pharmacol. Exptl. Therap. 42 : 27.   1931.
8. GRUBER, C. M. and GRUBER, C. M.   J. Pharmacol. Exptl. Therap. 72 : 176.   1941.
9. GRUBER, C. M., GRUBER, C. M., and COLOSI, N. A.   J. Pharmacol. Exptl. Therap. 63 : 215.   1938.
10. GRUBER, C. M., SCHOLTEN, R., DE NOTE, A., and WILSON, J. F.   J. Pharmacol. Exptl. Therap. 56 : 341.   1936.
11. HUSTON, M. J., MARTIN, A. W., and DILLE, J. M.   Arch. int. pharmacodynamie, 74 : 46.   1947.
12. KNOWLTON, G. C. and HINES, H. M.   Am. J. Physiol. 120 : 757.   1937.
13. LISSAK, K., DEMPSEY, E. W., and ROSENBLUETH, A.   Am. J. Physiol. 128 : 45.   1939.
14. NICHOLAS, J. S. and BARRON, D. H.   J. Pharmacol. Exptl. Therap. 46 : 125.   1932.
15. SWANSON, E. E.   J. Pharmacol. Exptl. Therap. 46 : 387.   1932.
16. SWANSON, E. E. and FRY, W. E.   J. Am. Pharm. Assoc. Sci. Ed. 26 : 1248.   1937.
17. TOWER, S. S.   Physiol. Revs. 19 : 1.   1939.
18. VAN HARREVELD, A.   Am. J. Physiol. 150 : 541.   1947.

THE GASTRIC RESPONSE OF MAN TO AN ACID TEST MEAL[1]

By Gordon E. Bermak and J. Doupe

Abstract

The gastric secretory response to the instillation of 300 ml. of 0.5% hydro-chloric acid was investigated 30 times in eight subjects by withdrawing six samples at 20 min. intervals and measuring the acid concentration. In six experiments duodenal regurgitation was prevented by constant duodenal suction. As this did not delay the fall in acidity ordinarily observed it was concluded that regurgitation was not essential for this process. In 14 experiments phenol red was added to the instilled solution and the amount of dilution was measured. It was found that on the average the decrease in concentration of the dye was the same as that of the acid although there were frequent small discrepancies. It was concluded that instillation of acid into the human stomach stimulates one or more secretions whose net effect is to reduce the acidity by dilution.

Introduction

It is obvious that following a rise of gastric acidity some process must take place to restore the acidity to the resting level. At one time it was generally assumed that duodenal regurgitation was the major factor but in 1924 Baird *et al.* (4) demonstrated, by aspirating the duodenal contents, that regurgitation was not essential to the process. In 1928 MacLean and Griffiths (10) presented further evidence indicating that in the human a diluting fluid of neutral character is secreted by the stomach and is chiefly responsible for the reduction of acidity. This denial of the importance of duodenal regurgitation prompted Apperly to review a former study (1) concerning the fate of an acid solution placed in the stomach. He and Norris (3), using an acid solution containing an indifferent substance to serve as an index of dilution, obtained results that were interpreted to mean that neutralization as well as dilution usually takes place and that the neutralizing fluid originates in the duodenum. Nevertheless, the concept of a purely gastric mechanism has continued to obtain support (7, 9, 13). For this reason and because of the potential usefulness of the acid instillation procedure for the investigation of the secretory functions of the stomach (2, 6, 8) it appeared worthwhile to repeat some of the experiments of Apperly and Norris in combination with the aspiration procedure of Baird *et al.*

Method

The acid reduction test of Elman (5) was used. It consisted of aspirating the fasting gastric contents, instilling 300 ml. of 0.5% hydrochloric acid, and withdrawing 20 ml. samples for analysis at 20 min. intervals for 100 min. Precautions against the swallowing of saliva were taken and the gastric contents were mixed by a pumping action of the syringe before each sample was removed.

In some instances the duodenum was drained throughout the test by the use of a double tube, one lumen of which connected the duodenum to a collecting flask at a pressure of minus 20 cm. water. The tube was considered to be in the duodenum as soon as an alkaline clear amber fluid was withdrawn. The maintenance of this position was confirmed by the continuous flow of a clear, heavily bile stained fluid from the tube varying in amount in different experiments from 350 to 450 ml. In about half the experiments 6 mgm. of phenol red was added to the acid solution. Penner *et al.* (11) have shown that for some purposes the dye method is invalid but in the present experiments, which are not concerned with water absorption, it appears applicable.

Samples were analyzed for free acidity by titrating with $0.1 N$ sodium hydroxide using Topfer's reagent. The concentration of the phenol red was measured with an Evelyn colorimeter using the method of Shay *et al.* (12). In some experiments, particularly those with duodenal drainage, the stomach was empty at 60 or 80 min. so the later samples could not be obtained.

The subjects consisted of eight healthy laboratory workers ranging in age from 19 to 40 years.

Results

The results are presented in Table I. The 12 experiments in which the simple test procedure was alone performed have been placed together in Group *A*. Bile was noted in most of the samples. Group *B* contains the experiments in which phenol red was added to the test solution. The color of the dye obscured bile if it was present. Groups *C* and *D* contain the experiments in which duodenal drainage was performed and differ from each other only in that phenol red was used in Group *D*. No bile was seen in the samples of these groups. Inspection of the data and the average values indicates that the decline in acidity was essentially similar in all the groups. Also presented in the table are the results concerning the degree of dilution undergone by the test solution. For comparison with the acid values, the data pertaining to dilution have been expressed as the normality of acid that would have resulted had the change in concentration of the dye been caused by the addition of a neutral unbuffered solution. To avoid the distortion likely to be introduced by any remaining fasting contents, the calculations were based on the equivalence of the acid and dye concentration in the initial sample rather than in that of the instilled solution. It will be seen that the observed acid values corresponded closely with the calculated values but that there were many minor discrepancies in both directions.

Discussion

If one disregards the possibility that acid may be absorbed in the stomach these results should be applicable to the problem of the site of origin and the nature of the secretion acting on the instilled acid. In regard to the first, the method of preventing regurgitation of duodenal contents would be entirely acceptable only if it did not alter other factors. That they were altered in

TABLE I

CHANGE IN NORMALITY OF AN ACID SOLUTION PLACED IN THE STOMACH

(Figures in parentheses show difference between the measured decline in normality and that predicted from dye dilution measurements. Plus values suggest an alkaline secretion)

Exptl. group	Subject	Acidity initial sample, normality HCl (× 1000)	Decrease in normality from value of initial sample (× 1000)				
			20 min. sample	40 min. sample	60 min. sample	80 min. sample	100 min. sample
A	Mr. B.	115	5	20	32	48	55
	Mr. B.	116	18	24	32	43	81
	Mr. B.	124	12	24	35	49	73
	Mr. B.	109	3	9	19	30	53
	Mr. B.	122	10	21	43	66	67
Acid alone	Mr. D.	115	16	27	60	67	70
	Mr. D.	126	11	26	35	52	64
	Mr. D.	130	16	30	46	55	69
	Mr. D.	132	19	23	52	75	82
	Mr. D.	126	6	18	29	66	79
	Mr. D.	130	20	44	49	66	86
	Mr. M.	124	20	40	58	—	—
	Mean:	122	13	25	41	51	65
B	Mr. B.	122	3 (0)	27 (+ 2)	26 (+ 8)	54 (+11)	74
	Mr. D.	131	5 (− 6)	8 (− 7)	17 (− 3)	27 (− 1)	41 (− 1)
	Miss B.	117	7 (− 3)	17 (+ 2)	29 (+ 1)	40 (+ 2)	48 (+ 3)
	Miss B.	125	16 (− 2)	30 (+ 7)	48 (+ 5)	57 (+ 7)	73
	Miss B.	123	10 (− 2)	18 (+ 2)	30 (− 2)	42 (+ 2)	55 (− 7)
Acid plus dye	Miss B.	126	12 (+ 2)	51 (− 7)	60 (0)	91 (− 1)	74 (+ 3)
	Mr. A.	105	0 (+11)	18 (+ 2)	76 (− 3)	—	—
	Mr. A.	127	26 (− 9)	63 (− 6)	114 (+ 2)	—	—
	Mr. A.	118	7 (+ 1)	26 (0)	45 (+12)	—	—
	Mr. A.	116	+1 (− 5)	15 (0)	42 (+ 5)	—	—
	Miss P.	121	14 (− 4)	24 (+ 4)	37 (+ 4)	60 (+11)	72 (− 4)
	Mean:	121	9 (− 1)	27 (0)	47 (+ 3)	53 (+ 3)	62 (0)
C	Mr. B.	120	4	10	23	74	—
	Mr. B.	119	13	22	31	47	50
Acid plus drainage	Mr. C.	124	19	52	98	—	—
	Mr. P.	124	9	31	42	—	—
	Mean:	122	11	29	48	60	—
D	Mr. B.	113	+2 (− 7)	29 (+12)	48 (+ 8)	68 (+14)	—
	Miss B.	131	15 (− 2)	26 (+ 4)	31 (+ 2)	37 (− 3)	44 (−11)
Acid plus dye plus drainage	Mr. A.	114	8 (− 3)	36 (+ 3)	101	—	—
	Mean:	119	8 (− 4)	30 (+ 6)	60 (+ 5)	52 (+ 5)	—

these experiments is shown by the faster rate of the emptying of the stomach when the duodenum was drained. Thus it cannot be stated that duodenal regurgitation had no effect at ordinary rates of gastric emptying. It is evident, however, that regurgitation was not essential for the reduction of the acidity of an acid test meal.

Regarding the nature of the secretion a comparison of its effects on the concentration of the dye and on the acidity shows that on the average its reaction must have been nearly neutral. However, the many discrepancies can only be explained by attributing them to technical limitations or to the action of both alkaline and acid secretions. Inspection of the average results reported by Apperly and Norris (3) shows that they too found an excellent agreement between dilution and the reduction of acidity. Even in the groups in which they were impressed by a difference their results show that the discrepancy was due to a factor operating only in the first 15 min. of the experiments. It is possible to conclude, therefore, that ordinarily the acidity of an acid test meal is reduced chiefly by dilution. It is not possible to state with any certainty whether this diluting fluid represents a single secretion or a combination of an alkaline and an acid secretion. The latter view has been upheld by Maclagan (9) and is supported by the varying discrepancies reported here.

Clearly, the present results support the contention of MacLean and Griffiths (10) that the acidity of the gastric contents is reduced by the diluting action of a neutral secretion of the stomach. However, owing to defects in available methods, such a conclusion cannot even yet be regarded as proved.

Acknowledgment

References

1. APPERLY, F. L. Brit. J. Exptl. Path. 7 : 111. 1926.
2. APPERLY, F. L. Am. J. Digestive Diseases Nutrition, 4 : 91. 1937.
3. APPERLY, F. L. and NORRIS, J. H. J. Physiol. (London), 70 : 158. 1930.
4. BAIRD, M. M., CAMPBELL, J. M. H., and HEARN, J. R. B. Guy's Hosp. Repts. 74 : 23. 1924.
5. ELMAN, R. Surg. Gynecol. Obstet. 49 : 34. 1929.
6. ELMAN, R. and MACLEOD, J. W. Am. J. Digestive Diseases Nutrition, 2 : 21. 1935.
7. GOLDBERG, S. L. Arch. Internal Med. 49 : 816. 1932.
8. LEVY, J. S. Ann. Internal Med. 7 : 1244. 1934.
9. MACLAGAN, N. F. Quart. J. Med. 3 : 321. 1934.
10. MACLEAN, H. and GRIFFITHS, W. J. J. Physiol. (London), 65 : 63. 1928.
11. PENNER, A., HOLLANDER, F., and POST, A. Am. J. Digestive Diseases Nutrition, 7 : 202. 1940.
12. SHAY, H., GERSHON-COHEN, J. MUNRO, F. L., and SIPLET, H. J. Lab. Clin. Med. 26 : 732. 1941.
13. SHAY, H., KATZ, A. B., and SCHLOSS, E. M. Arch. Internal. Med. 50 : 605. 1932.

THE REACTION OF VARIOUS TISSUES TO IMPLANTS OF A COLLAGEN DERIVATIVE[1]

By Arthur F. Battista

Abstract

This study deals with the histological reaction of subcutaneous connective tissue, muscle, peritoneum, nervous tissue, and bone to implants of a collagen derivative obtained from bovine bone, which has been called Collatissue A. The tissue response was in two stages, first, a fibroblastic proliferation about the implant and second, an infiltration by small and large mononuclear cells. Capillary proliferation occurred around the implant. The material gradually lost its normal histological structure, became amorphous, and was replaced by fibrous tissue. There was no polymorphonuclear cell response and giant cells appeared rarely. Silk, catgut, and tantalum foil were used as control materials. They induced a typical foreign body response, which included polymorphonuclear and giant cells. The antigenic properties of Collatissue A were studied to a limited extent with negative results. Collatissue A has certain physical properties, such as tensile strength and flexibility, that suggest possible surgical applications of this material.

Introduction

Surgeons have long sought for a material that would elicit very little foreign body response and that would be transformed eventually into living tissue. Such a substance might be termed a 'physiological' plastic material. The difficulty of procuring such a substance has deflected much investigation into the channel of alloplastic materials such as celluloid, various metals, paraffin, vaseline, latex, amber, and ivory. The requirements are nonabsorbability, resistance to deleterious alteration by tissue fluids, chemical inactivity, and freedom from properties that would initiate early or late tissue reaction. Each of the above-mentioned substances, as well as many others, has had its trial and supporters. The thermoplastic synthetic resins such as methyl methacrylate (Lucite and others) are the most recent materials to be studied from the point of view of surgical application.

The physical and chemical properties of collagen make it a hypothetical source of a 'physiological' plastic substance that might be utilized by the tissue elements, especially fibroblasts, and become part of the living tissue. The studies that are reported at this time are directed primarily at the histological aspects of this problem.

Historical Review

Relatively few studies of the biological properties of collagen or its denatured products (excluding gelatin) have been published. The earliest reports are descriptions of the use of decalcified chicken bone and other tubular bones as surgical drains. Protective cuffs of similar material were used about the suture line of previously sectioned nerves (12, 16, 30, 36, 1). In 1880 Senn

(33) employed antiseptic decalcified bone as an aid in the healing of aseptic bone cavities. The great difficulty was to obtain a sterile field. The material was a protein and served as an ideal medium for the growth of bacteria until it was vascularized and invaded by connective tissue (6).

Barth, in 1894 (3, 4) grafted decalcified bone to bone in the host and obtained only fibrous connective tissue union. However, Senn (33) stated that calcification did occur in some animal experiments where he had used a similar material in contact with bone.

Nageotte (26, 24, 23) isolated collagen from rat tails by a process of extraction by dilute acetic acid and precipitation with salt solution. He studied the behavior of this material in tissue cultures. He also grafted animal, human cadaver, and human living tendons into patients and found them to be tolerated well (21). Finally, Nageotte investigated the formation of collagen in tissues and isolated different fractions (27, 28). Pullinger and Pirie (31) isolated collagen from ox cornea by the Bergmann method and studied the reaction of rabbit subcutaneous tissue to the product. They concluded that collagen elicited a mild reaction that resembled chronic inflammation. They attributed this response to the insolubility of the material rather than to a chemically induced foreign body reaction.

Feriz (8, 9) studied the reaction of living tissue to a material made of collagen derived from beef tendón. He stated that the material (which was called Brocatamp) was assimilated and appeared nonirritating to the surrounding tissue when implanted in rabbits. His studies indicated that the material obtained from tendon caused less tissue reaction than did catgut or silk. Weiss and Taylor (37) employed collagen tubes in the study of nerve regeneration across gaps. No description of the preparation of these collagen tubes was given except that the tubes were "spun from fibers of dissolved and reprecipitated bovine collagen by a process developed at the Massachusetts Institute of Technology under the direction of Dr. F. O. Schmitt." Tanned and untanned tubes were prepared. They used the untanned collagen tubes in the nerve regeneration experiments but the tubes were resorbed too quickly and did not prevent loss of liquid or ingrowth of fibrous tissue. They also stated that they "have made comparative studies of the relative merits of the various collagen sleeves in ordinary nerve splicing experiments, but have been unable to reach final conclusions because of the great number of variables involved (variability of the preparations, animals, operative incidents, etc.)".

Materials and General Methods

Various authors have proposed different methods of obtaining collagen (28, 24, 9, 10). It was difficult to procure, by these methods, material that had the physical properties desired without greatly altering its chemical nature. The material used in this investigation was obtained by treating beef bone with dilute hydrochloric acid until all trace of calcium was removed. As a matter of convenience it was called Collatissue A with the thought that other collagen derivatives may form the subject of future investigations.

Bacteria and spores were destroyed in the processing, and the material was stored in 30% ethyl alcohol. It was transferred to 80% alcohol for several days before use. The material was washed thoroughly with sterile water before implantation. Since it is hydrophilic, it is likely that most of the alcohol, except that which was adsorbed, was removed. Very few infections have been encountered, and none have been related to the material. Bacteriological cultures were made of a few samples and have been negative.

Cats were used throughout the experiments since they respond well to multiple short procedures such as repeated removal of implants after varying periods of time. Intraperitoneal nembutal anesthesia was used for the implantation operation, ether for the subsequent removal procedures. Scrupulously sterile technique was the rule.

Specific Methods and Results

Series I.   The Reaction of Subcutaneous Connective Tissue (and Skin) to Implants of Collatissue A

Procedure

A dorsal midline incision was made, an artery forceps passed far laterally under the skin, and a small subcutaneous pocket made by opening the forcep. Three pieces of Collatissue A, each 2 cm. × 1 cm. × 2 mm., were deposited in the subcutaneous space. The final position of the implants was on the ventrolateral aspect of the animal's body wall, far removed from the incision and its sutures. A midline abdominal incision was avoided because of the increased possibility of infection of the wound during the postoperative period.

Silk (Deknatel 6-0), catgut (type A Scanlan Plain No. 2), and tantalum foil (0.0005 in. thick) were implanted in the same manner for comparative studies.

Nine implants of Collatissue A, with surrounding tissue, were removed under sterile conditions at intervals varying between 13 and 237 days. For comparative studies there were three specimens of implanted silk (7, 20, and 28 days), three of catgut (7, 28, and 45 days), and four of tantalum foil (7, 20, 28, and 45 days). The material was fixed in Zenker–formol and stained with haematoxylin and eosin and by Mallory's method.

Observations

The reaction of subcutaneous tissue to implants of Collatissue A was relatively slight. The initial response consisted of a proliferation of connective tissue elements adjacent to the implant. This response was well developed by the 13th day. The amount of fibroblastic proliferation varied from one animal to another but in all cases a well defined capsule was formed about the implant (Figs. 1, 2). In some of the specimens implanted for long periods there was less capsular tissue present than in those of short duration. The capsule consisted of layers of fibroblasts of typical cytological structure. In later stages the fibroblasts were seen to invade the surface of the implant.

The later response of subcutaneous tissue to Collatissue A was an infiltration of adjacent tissue by small and large mononuclear cells. Very few cells of this type were present in the early stages. The time of first appearance was variable with a maximal infiltration 50 to 60 days after implantation. The mononuclear cells tended to occur in groups of 10 to several hundred. The presence of a few of these cells in the central portion of the implant attested to their amoeboid properties. Giant cells were encountered very rarely. Capillary proliferation was evident adjacent to the implant.

Collatissue A specimens that had been implanted for varying periods lost their typical histological structure in certain parts, and became amorphous. However, specimens that were in subcutaneous tissue for 237 days were readily palpable through the skin and the implant had maintained its shape and tensile strength (Fig. 2).

In contrast with the mild reaction to Collatissue, implantation of silk, catgut, and tantalum foil resulted in a typical foreign body reaction. In addition to the fibroblastic reaction, capillary proliferation, and appearance of large and small mononuclear cells, there were large numbers of polymorphonuclear cells and frequent giant cells (Figs. 4 and 5).

Series II.   The Reaction of Muscle to Implants of Collatissue A

Procedure

Pieces of Collatissue A were embedded in the substance of the longus colli and longissimus dorsi muscles. The precautions followed in the case of subcutaneous implants were carefully observed. Silk, catgut, and tantalum foil were used for comparative studies. Specimens of Collatissue were removed at intervals varying between 3 and 149 days. The reaction of muscle tissue to silk, catgut, and tantalum foil after 7, 18, 28, and 45 days was studied.

Observations

The sequence of events following implantation of Collatissue A in muscle was very similar to the reaction in subcutaneous tissue (Fig. 3). The implant became encapsulated but the capsule was thinner than that produced in subcutaneous tissue (Fig. 1). The fibroblasts were more variable in orientation than in the capsule produced in the subcutaneous location. The fibroblasts invaded the substance of the implant and in the older specimens the surface areas of the Collatissue became amorphous and broken up. In one specimen of 35 days'-duration, portions of the implant had disappeared, being replaced by fibrous tissue of lesser volume than the original Collatissue. In other specimens, which had been in muscle for longer periods of time, the implant remained relatively intact, demonstrating the great variability in the rate of disappearance of the implant in muscle tissue. Several factors are likely to

influence the fate of the implant. The vascularity of the tissue and age of the animal are possible controlling factors. The implant appears to be altered more quickly in the young growing animal.

Large and small mononuclear cells were relatively numerous adjacent to the implant. Very few polymorphonuclear cells were seen and giant cells were encountered only occasionally. Catgut, silk, and tantalum foil, however, called forth a typical foreign body reaction. The polymorphonuclear response to silk and catgut was profuse, but somewhat less in the case of tantalum foil. Many giant cells were present about the catgut implant, some multinucleated to such an extent that they appeared as a syncytium. Occasional eosinophiles were encountered. Catgut, silk, and tantalum all incited a fibroblastic response and capillary proliferation. In general, the tissue reaction to tantalum was less marked than to silk or catgut but still much greater than to Collatissue.

B..........*Bone.*		E..........*Area in Fig. 6 enlarged and shown in Fig. 7.*	
Ca..........*Capsule.*		F..........*Fibroblastic tissue.*	
Ce..........*Cellular elements.*		G..........*Catgut.*	
Co..........*Collatissue A implant.*		M..........*Muscle.*	
D..........*Dermis.*		P..........*Panniculus carnosus muscle.*	
Dc..........*Cranial dura mater surface.*		T..........*Position of tantalum foil implant.*	

FIG. 1.   *Series I.   The reaction of subcutaneous tissue to implants of Collatissue A. Specimen No. 2 (17 days) haematoxylin-eosin (H.-E.) stain.   A well defined capsule (Ca) is seen adjacent to the surface of the implant towards the dermis (D). It is definitely thicker than the capsule in earlier sections.   × 200.*

FIG. 2.   *Series I.   The reaction of subcutaneous tissue to implants of Collatissue A. Specimen No. 9 (237 days) H.-E. stain.   The position of the implant (Co) is visible deep to the dermis (D).   Capsules (Ca) are present about the surfaces of the implant.   Relatively little change in the structure of the implant is observed. The edges in contact with the capsules are not broken up to any great degree.   × 30.*

FIG. 3.   *Series II.   The reaction of muscle to implants of Collatissue A. Specimen No. 9 (129 days) H.-E. stain.   The position of the implant (Co) is seen between the muscle tissue (M).   Capsule formation (Ca) is visible on each surface of the implant.   × 30.*

FIG. 4.   *Series I.   (Subcutaneous tissue) control catgut. Specimen No. 2 (28 days) H.-E. stain.   The position of catgut (G) is seen. Cellular infiltration (Ce) about the implant is visible.   × 30.*

FIG. 5.   *Series I.   (Subcutaneous tissue) control tantalum foil. Specimen No. 1 (seven days) H.-E. stain.   The tantalum foil implant (T) elicits a foreign body tissue response.   A definite capsule formation (Ca) is observed. Many cellular elements (Ce) are present, especially polymorphonuclear cells. × 125 approximately.*

FIG. 6.   *Series VII.   The reaction of bone and cranial dura mater to Collatissue A. Specimen No. 5 (230 days) H.-E. stain.   The position of Collatissue A (Co) in relation to bone (B) is seen.   × 12.*

FIG. 7.   *Series VII.   The reaction of bone and cranial dura mater to Collatissue A. Specimen No. 5 (230 days) H.-E. stain.   This photomicrograph is area (E), Fig. 6, seen under higher magnification.   The junction of bone and Collatissue A implant is shown.   Fibroblastic elements (F) form this junction.   Large and small mononuclear cells constitute the limited cellular infiltration.   × 90.*

PLATE I
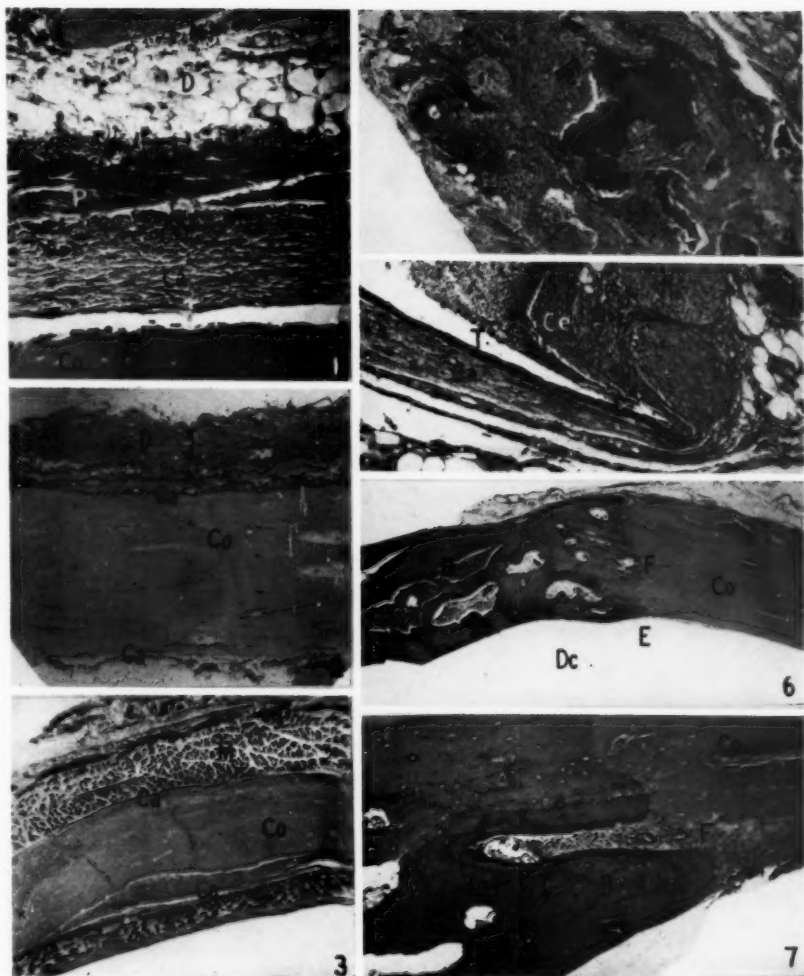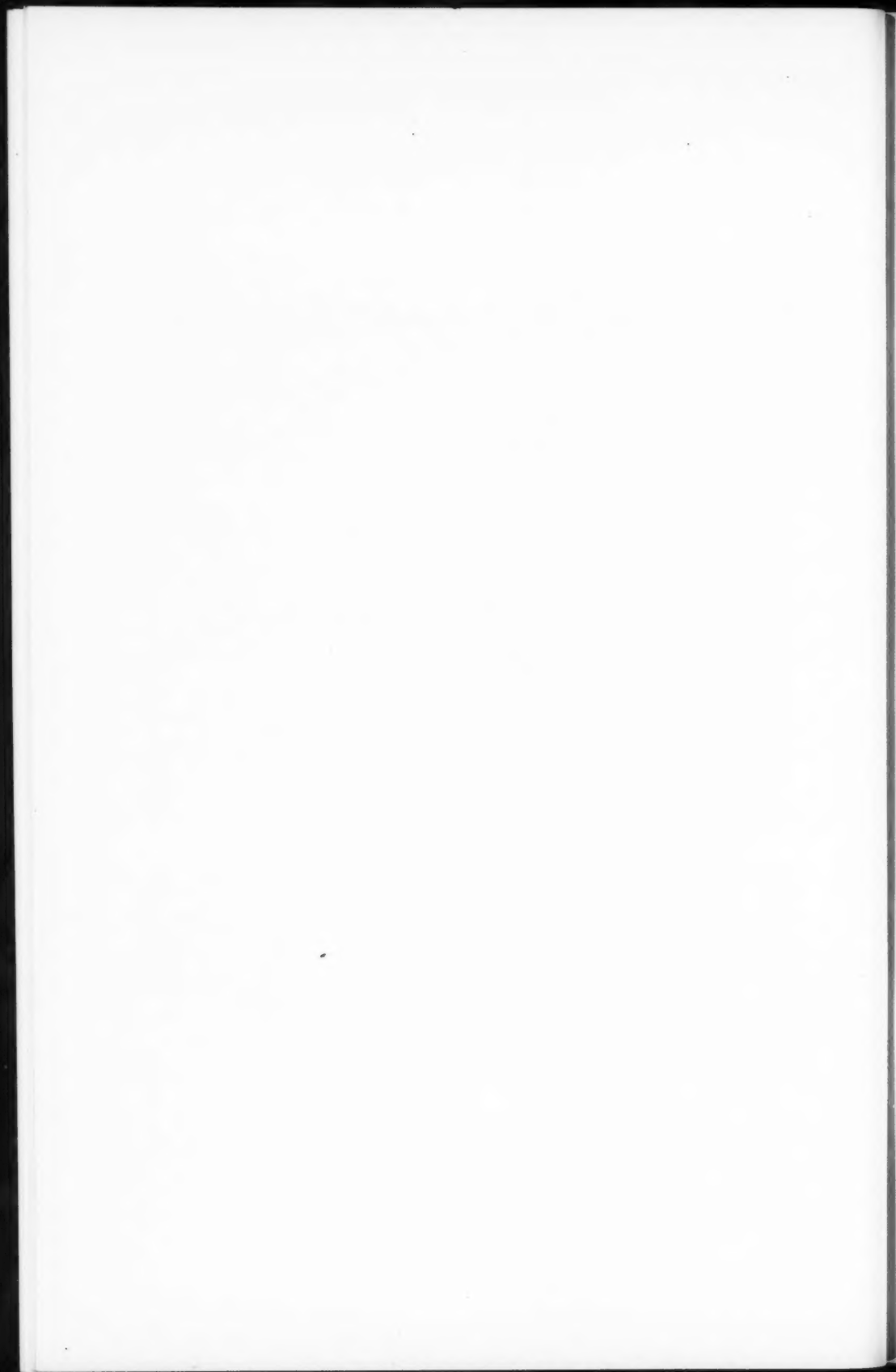
Series III. The Reaction of Peritoneum to Implants of Collatissue A

Procedure

The ventral parietal peritoneum was exposed and an area measuring 2 cm. square removed. The peritoneal defect was filled in by a thin sheet of Collatissue, 2 mm. in thickness. It was held in place by tucking the edges between the bordering peritoneum and overlying muscle thus avoiding sutures. The fascia and skin were closed with silk. Six specimens varying in duration between 7 and 158 days were obtained.

For comparison, in a single animal a large defect was created in the parietal peritoneum and the viscera left in contact with the abdominal muscles.

Observations

There was very little tissue reaction to the peritoneal replacement. A thin fibrous capsule developed on its inner surface, consisting of typical fibroblasts arranged in a linear manner. In some specimens the omentum was adherent to the implant but it stripped away easily. In no case was there adherence of the intestine. A mild cellular infiltration of large and small mononuclear cells occurred with an occasional polymorphonuclear and giant cell. In the control animal, the intestine and omentum were bound firmly by adhesions to the muscle of the abdominal wall.

Series IV. The Reaction of Smooth Muscle of the Intestinal Wall to Collatissue A

Procedure

A small sheet of Collatissue 1 cm. × ½ cm. × 2 mm. was inserted between the muscle layers of the small intestine. The serosa was sutured with fine 6-0 silk. Two specimens, of 17 and 128 days duration were obtained.

Observations

The characteristic response to Collatissue A was observed. A fibroblastic capsule surrounded the implant, and the cells invaded the material. The surrounding tissue was infiltrated with large and small mononuclear cells, an occasional polymorphonuclear cell, and a rare giant cell. Capillary proliferation occurred in the capsule. The connective tissue of the submucosa adjacent to the implant appeared to have increased in thickness. The mucosa remained normal. There was no evidence of intestinal disturbance in the animal during life.

Series V. The Reaction of the Omentum to Collatissue A

Procedure

A piece of Collatissue 1 cm. × ½ cm. × 2 mm. was wrapped in omentum and held in place by a fine silk suture through one end of the implant. Two specimens were obtained at intervals of 17 and 128 days.

Observations

The omentum is considered a delicate tissue that responds to the slightest irritation or foreign body stimulation. Implants of Collatissue A elicited a reaction similar to that in other tissues. The usual fibrous capsule developed with invasion of the surfaces of the implant by fibroblasts. In the specimen of longer duration the Collatissue lost its normal histological structure near the surface and acquired an amorphous, moth-eaten appearance. In some areas the implant had undergone disintegration and disappeared. A cellular infiltration with large and small mononuclear cells occurred earlier than in other locations. Occasional polymorphonuclear and giant cells were seen.

Series VI. The Reaction of Cerebral Cortex and Cranial Dura Mater to Collatissue A

Procedure

A rectangular bone flap was reflected over the frontoparietal area of the skull to expose a portion of both cerebral hemispheres. An area of dura mater 1 cm. square was removed over one hemisphere. The exposed cortex was traumatized with the point of a scalpel and by the actual removal of cortical tissue by suction. There was much bleeding. A piece of very thin Collatissue A was placed over the traumatized cortex, filling in the dural defect. Three specimens, at intervals of 108, 113, and 168 days, were obtained.

For comparison, the dura was replaced by tantalum foil in several instances. In one animal the dural defect was not filled in, but the traumatized cortex was left in contact with the replaced bone flap in the case of one hemisphere, while the dural edges were drawn together over the traumatized cortex of the opposite hemisphere by two fine 6-0 silk sutures.

Observations

No adhesions were observed between the cortex and the Collatissue A dural replacement. In a few instances where the cortex had been extensively traumatized and partially removed by suction, a few strands of connective tissue were present. They stripped away very easily. The replacements were firmly fixed to the dura at the edge of the defect. The side of the implant in contact with bone bore a thin capsule of fibroblastic tissue. A few fibroblasts were seen on the inner surface but no capsule developed. The number of fibroblasts on the cerebral surface was greatest in the 168-day specimen. There was slight invasion of surrounding tissue by large and small mononuclear cells. Polymorphonuclear and giant cells were rarely seen.

In the case of tantalum foil implants the cortex was firmly adherent to the capsule that had developed on the cerebral surface of the implant. In some instances the adherent cortical tissue came away with the tantalum when the latter was reflected. Strong adhesions were present between bone and cortex where the dura had been extirpated and the cortex exposed to the bone flap.

Series VII. The Reaction of Bone and Cranial Dura Mater to Collatissue A

Procedure

A defect 1 cm. in diameter was made with a trephine over the fronto-parietal area of the skull. The defect was filled by a disc of Collatissue A.

In other cases a rectangular bone defect was filled in with Collatissue A.

Five specimens, the periods varying between 20 and 230 days, were available for study.

Observations ·

In the 230th day specimen the Collatissue replacement remained intact. The fibrous tissue of the scalp formed a capsule on the outer surface of the implant to which the scalp was firmly fixed. There were no firm adhesions between the cranial dura mater and the Collatissue A, for the dura mater stripped away as freely as it does from bone. The implant was firmly adherent to the surrounding bone edge (Figs. 6 and 7). In older specimens it appeared that the osteocollagenous fibers of the bone were continuous with the fibers of the implant. The two materials could be differentiated by the marrow spaces of the bone and the normal histological characteristics of Collatissue. Very few cellular elements, mostly large and small mononuclear cells, were present at any stage. Polymorphonuclear and giant cells were seen rarely.

Preliminary Studies of Antigenic Properties of Collatissue A

Several preliminary tests were made to determine the antigenic properties of Collatissue A. A sterile suspension (1½ cc.) of the material (particles of almost colloidal size) was injected intraperitoneally into two mature guinea pigs. A third guinea pig received 1 cc. of the suspension intravenously and three rabbits received 2 cc. by the same route. After a period of 16 days the animals received 1 cc. of the same suspension intravenously. No reaction occurred. In a cat, a member of a series not reported upon at this time, the thorax on one side was packed with Collatissue A in order to collapse the lung. After a period of 12 months the animal received 1 cc. of a Collatissue A suspension intravenously with no immediate or delayed effects. No reaction occurred following repeated intravenous injections in this animal over a period of a month. These experiments suggest that Collatissue A has no, or very limited, antigenic properties. More extensive and precise studies are planned to determine the immunological properties of Collatissue A and similar collagen derivatives.

Discussion

Several mechanisms may be proposed for the mild response to Collatissue A when compared with silk, catgut, and tantalum foil. Some workers maintain that collagen derived from different mammals is similar chemically so that a denatured product of collagen would not be foreign to the tissues of different animals. Pullinger and Pirie (31) stated that the response of subcutaneous

tissue in the rabbit to collagen derived from ox cornea resembled the tissue reaction seen in chronic inflammation.   There was a predominance of lymphocytes, macrophages, and mature connective tissue fibers.   They attributed the comparatively mild reaction to the insolubility of collagen and referred to the absence of features considered to be typical of a foreign body reaction.

The mechanism by which the disappearance of Collatissue A is accomplished, when this does occur, appears to be complex.   The virtual absence of polymorphonuclear and giant cells, as compared with catgut and silk, tends to preclude a phagocytic process.   The change from a typical architecture to an amorphous material suggests an enzymatic attack on the material resulting in increased solubility and assimilation of the altered collagen.   The numerous mononuclear cells and young growing fibroblasts may play a part in this process.   It is of interest to note that Feriz (8, 9) studied the fate of a commercial material made of collagen derived from beef tendon when placed in tissues of the rabbit.   He stated that the material was partly absorbed by lytic activity of ferments, partly removed by phagocytosis, and partly organized either by direct infiltration of connective tissue cells or by the formation of granulation tissue.

The comparative observations on the fate of catgut, silk, and tantalum foil show how intimately polymorphonuclear and giant cells are associated with the foreign body reaction.

The first visible evidence of tissue reaction to Collatissue A is a fibroblastic response.   The degree of this response varies considerably in the same tissue, and is not intimately related to the period during which the implant was embedded.   Both the fibroblastic response and the amount of absorption of the material varies considerably in different tissues.   There are probably multiple factors involved in this variation.   Differences in the vascularity of various tissues is likely to be an important factor.   It seems possible that the degree of participation of Collatissue A in the function of the tissue in which it is implanted may be important.   The implant is readily absorbed in muscle in which it can serve no useful function.   On the other hand, Collatissue A that replaced a peritoneal defect survived and developed a well defined fibrous capsule on its visceral surface, thus performing the useful function of repairing a peritoneal defect.   In this connection the results obtained by replacing a portion of the Achilles tendon in a dog by a strip of Collatissue A is of considerable interest.   After removal of a plaster cast at the end of three weeks the animal had normal use of the limb in which the repair had been carried out.   The dog was observed for a period of eight months.   It was impossible to detect any difference in the two limbs when the dog ran and jumped.   Bone grafts are known to behave in a similar manner.   Bone implanted in muscle tends to undergo decalcification and to be absorbed or it may become encapsulated by fibrous tissue.   Bone grafted into bone becomes incorporated in the living dynamic tissue of its new environment.

The experience gained in this study of the biological properties of Collatissue A commends its consideration as a source material in the plastic repair of surgical defects and numerous other surgical procedures.   A more extensive series of animal experiments is planned to inquire more specifically into its practical applications.   Further studies of the biochemical, physical chemical, physical, and immunological properties of collagen derivatives are also required.   The final evaluation of the practical value of Collatissue A in surgery can only be obtained from actual clinical trials.

Summary of Results

The various tissues of the cat that were studied in this investigation showed relatively little reaction to implants of Collatissue A.   The response may be considered in two stages.   There is, first, a proliferation of fibroblasts, derived from the surrounding connective tissue around the implant to form a capsule. Secondly, a cellular infiltration consisting of large and small mononuclear cells occurs.   The latter resemble lymphocytes cytologically.   Very few polymorphonuclear cells are seen and in some specimens there are none. Giant cells are rarely encountered.   The cellular infiltration tends to be accompanied by a change in the histological structure of the implant.   The material becomes amorphous and stains more lightly, these changes occurring mainly near the surface of the implant.   Collatissue changed in this way is replaced by fibrous tissue.

The implanted specimens of silk, catgut, and tantalum foil called forth a typical foreign body response.   In addition to the mononuclear cells there were large numbers of polymorphonuclear cells and numerous giant cells.   In the case of silk and catgut especially, the giant cells were so large and the nuclei so numerous that they took on the appearance of a syncytium.

Preliminary experiments failed to detect any antigenic properties for Collatissue A.

Acknowledgments

References

1. AUFFENBERG, F. v.   Arch. klin. Chir. 82 : 615.   1907.
2. BAITSELL, G. A. and MASON, K. E.   Am. Rev. Tuberc. 29 : 587.   1934.
3. BARTH, A.   Arch. klin. Chir. 48 : 466.   1894.
4. BARTH, A.   Arch. klin. Chir. 86 : 859.   1908.
5. BERGMANN, M. and STEIN, W. H.   J. Biol. Chem. 128 : 217.   1939.
6. DEAVER, J. B.   Med. News, 4 : 714.   1889.

7. EVANS, R., COWDRY, E. V., and NIELSEN, P. E.   Anat. Record, 86 : 545.   1943.
8. FERIZ, H.   Surgery, 8 : 654.   1940.
9. FERIZ, H.   Surgery, 10 : 326.   1941.
10. FLORENCE, G. and LOISLEUR, J.   Bull. soc. chim. biol. 16 : 52.   1934.
11. FOOT, N. C.   Am. J. Path. 4 : 525.   1928.
12. GLUCK, T.   Arch. klin. Chir. 25 : 606.   1880.
13. HASS, G.   Am. J. Path. 16 : 549.   1940.
14. HASS, G. and McDONALD, F.   Am. J. Path. 16 : 525.   1940.
15. KNAGGS, J.   Biochem. J. 23 : 1308.   1929.
16. KOLLIKER, T.   Deut. Chir. 24*b*, p. 120.   1890.
17. LOWRY, O. H., GILLIGAN, D. R., and KATERSKY, M. E.   J. Biol. Chem. 139 : 793.   1941.
18. MACKIE, W.   Med. News, 57 : 202.   1890.
19. MILLER, A. G.   Lancet, 2 : 618.   1890.
20. MURRAY, R. W.   Lancet, 2 : 719.   1890.
21. NAGEOTTE, J.   Compt. rend. soc. biol. 95 : 669.   1926.
22. NAGEOTTE, J.   Compt. rend. soc. biol. 96 : 172.   1927.
23. NAGEOTTE, J.   Compt. rend. soc. biol. 96 : 464.   1927.
24. NAGEOTTE, J.   Compt. rend. soc. biol. 96 : 828.   1927.
25. NAGEOTTE, J.   Compt. rend. soc. biol. 97 : 559.   1927.
26. NAGEOTTE, J.   Compt. rend. soc. biol. 98 : 15.   1928.
27. NAGEOTTE, J.   Compt. rend. soc. biol. 104 : 156.   1930.
28. NAGEOTTE, J.   Compt. rend. soc. biol. 113 : 841.   1933.
29. NAGEOTTE, J.   Presse méd. 74 : 27.   1939.
30. POMERANCEW.   Zur Casuistik der Nervennaht Wratsch, no. 1, p. 26.   1900.
31. PULLINGER, B. D. and PIRIE, A.   J. Path. Bact. 54 : 341.   1942.
32. SCHMITT, F. O., HALL, C. E., and JAKUS, M. A.   J. Cellular Comp. Physiol. 20 : 11. 1942.
33. SENN, A.   Am. J. Med. Sci. n.s. 98 : 219.   1889.
34. SIZER, I. W.   Surgery, 121 : 231.   1945.
35. STEARNS, M. L.   Am. J. Anat. 66 : 133.   1940.
36. VANLAIR, C.   Arch. biol. (Liége), 3 : 379.   1882.
37. WEISS, P. and TAYLOR, C.   J. Neurosurgery, 3 (5) : 375.   1946.
38. WOLBACH, S. B. and HOWE, P. R.   Arch. Path. Lab. Med. 1 : 1.   1926.
39. WOLBACH, S. B. and HOWE, P. R.   J. Exptl. Med. 42 : 753.   1925.

ACETYLCHOLINE AND NEURONAL ACTIVITY

I. CHOLINESTERASE PATTERNS AND ACETYLCHOLINE IN THE CEREBROSPINAL FLUIDS OF PATIENTS WITH CRANIOCEREBRAL TRAUMA[1]

By Donald B. Tower and Donald McEachern

Abstract

Cerebrospinal fluid acetylcholine and cholinesterase determinations were made on 112 neurological and neurosurgical patients. Results on 15 patients with craniocerebral trauma and six psychiatric patients treated with electric shock convulsant therapy (E.C.T.) are reported in detail. Except for epileptics the only cerebrospinal fluid assays positive for acetylcholine occurred in the traumatic and E.C.T. groups, in which acetylcholine levels were 0.2 to 100+ μgm. %. In contrast to all other patients cerebrospinal fluid cholinesterases of traumatic and E.C.T. groups showed reversals of normal fraction patterns. These consisted of reduction of specific and increase of unspecified cholinesterase fractions, together with decrease of total cholinesterase activity. Acetylcholine levels and extent of cholinesterase fraction reversals paralleled the severity of cerebral damage, judged by clinical and electroencephalographic (E.E.G.) signs. Coma or semiconsciousness and depression of E.E.G. activity were associated with presence of acetylcholine and marked reversals of cholinesterase fractions in cerebrospinal fluids. In less severe cases and during recovery, when patients were disoriented and confused and E.E.G. activity was increased, cerebrospinal fluid acetylcholine decreased and disappeared, and cholinesterase values returned toward normal. Similar cerebrospinal fluid abnormalities were seen in patients after E.C.T. Changes in cerebrospinal fluid cholinesterase fraction patterns seem to be sensitive indications of extent of cerebral injury and progress toward recovery.

Introduction

In the course of studies on acetylcholine and cholinesterases in cerebrospinal fluids from 112 neurological and neurosurgical patients, interesting findings were obtained in the group with craniocerebral trauma. The literature on experimental studies of the physiology of craniocerebral trauma has been reviewed by Bornstein (3). He carried out animal experiments to investigate the role of acetylcholine in the post-traumatic state. He felt that there was a relationship of post-traumatic behavior, electroencephalographic abnormalities, and cerebrospinal fluid acetylcholine levels with the severity of concussion. He was able to reproduce these changes in unanesthetized animals by perfusion of the cortex with acetylcholine in concentrations of 1.0 to 10.0 μgm. %. With concentrations below 2.0 μgm. %, Bornstein (3) observed excitatory or synchronizing effects of acetylcholine on behavior and on the electroencephalograms (E.E.G.), while depressant effects appeared at higher concentrations.

We have reported in other papers the content and types of cholinesterases to be found in human cerebrospinal fluids (22) and the acetylcholine levels found in the same cerebrospinal fluid samples (8, 23). Three groups of

patients have been recognized.   (*a*) Normal individuals and patients with various neurological disorders: In this group cerebrospinal fluid cholinesterase values were normal and acetylcholine was absent from the cerebrospinal fluid. (*b*) Epileptic patients: In this group cerebrospinal fluid cholinesterase values were normal and acetylcholine was present in the cerebrospinal fluids (in amounts from 0.02 to 5.0 μgm. %).   (*c*) Craniocerebral trauma patients and patients treated with electric shock convulsant therapy (E.C.T.): In this group cerebrospinal fluid cholinesterase values were abnormal and acetylcholine was present in the cerebrospinal fluids in most instances.   Clinical and E.E.G. observations of the traumatic group indicated that we were seeing for the first time in human patients phenomena that Bornstein (3, 4) had described in experimental animals.*   No cerebrospinal fluid cholinesterase determinations were made by Bornstein (3, 4), so that abnormalities in the enzyme values reported here are the first to demonstrate their association with craniocerebral trauma.

Materials and Methods

Cerebrospinal fluid samples from 112 neurological and neurosurgical patients were examined for acetylcholine levels, cholinesterase values, and routine constituents.   Of the samples, 80% were obtained during pneumoencephalography, the remainder during ventriculography (4%) or lumbar puncture (16%).   Cell counts, Pandy tests, and protein determinations were carried out on all samples.   Cerebrospinal fluid cholinesterase activities and fraction characterizations (using acetylcholine (ACh), mecholyl (MeCh), and benzoylcholine (BCh) as substrates) were done by our modification (22) of the Warburg method of Ammon (1*a*), Odom *et al.* (18) and Nachmansohn and Rothenberg (17).   Cerebrospinal fluid samples for acetylcholine analysis were preserved by a modification (20) of the method of Babkin *et al.* (2) and later assayed by our modification of the method of Wait (24) on the isolated ventricle of *Venus mercenaria* (21).   Electroencephalograms (E.E.G.) were taken by conventional four-channel ink-writing recorders through the cooperation of the Department of Electroencephalography.

Results

1. *Cerebrospinal Fluid Acetylcholine and Cholinesterases*

Of the 112 patients studied 15 were cases of craniocerebral trauma and nine, cases treated by electric shock convulsant therapy (E.C.T.).   Only these 24 cases are considered at length in this paper.   The clinical diagnoses of these patients are included in Tables III and IV.

A total of 59 cerebrospinal fluid samples from 53 nonepileptic patients were assayed for acetylcholine.**   The results are summarized in Table I.

* *The inclusion of patients treated by electric shock convulsant therapy (E.C.T.) with cases of craniocerebral trauma may seem surprising.   We feel that the application of convulsive electric shocks to the cortex may be closely allied pathologically to a blow on the head (see Discussion).*

** *The results of acetylcholine assays on 60 cerebrospinal fluid samples from 57 epileptic patients are reported in a separate paper (8, 23).*

TABLE I

CEREBROSPINAL FLUID ACETYLCHOLINE ASSAYS IN 53 NONEPILEPTIC PATIENTS

Sample type	ACh positive		ACh negative		Total
Craniocerebral trauma patients	7	(35 %)	13	(65 %)	20
E.C.T. treated psychiatric patients	2	(33 %)	4	(67 %)	6*
Untreated psychiatric patients	0		4	(100 %)	4*
Others	2	(7 %)	28	(93 %)	30
Total	11	(18 %)	49	(82 %)	60
Subdural fluids	0		2		2
	11		51		62

* *One patient examined before and after E.C.T.*

It can be seen that 78% of the positive acetylcholine assays occurred in the traumatic groups of patients, while 65% of the negative acetylcholine assays were in the nontraumatic groups. In only two nontraumatic cases was acetylcholine found in the cerebrospinal fluid. One of these was a case of Arnold-Chiari malformation and the other a case with no central nervous system disease. With these two exceptions the nonepileptic cerebrospinal fluid samples positive for acetylcholine were restricted to cases of craniocerebral trauma (including E.C.T. cases).

Cerebrospinal fluid cholinesterase determinations were done on 111 samples from 102 patients. The results when correlated with the clinical diagnoses are summarized in Fig. 1. We have previously pointed out that there were no significant differences among the various diagnostic groups with the exception of the cases of craniocerebral trauma and those treated by E.C.T. (22). In most of the latter cases there was a tendency toward a lower rate of acetylcholine hydrolysis and a definite reversal of the usual fraction patterns, consisting of a relative decrease in the specific cholinesterase fraction (MeCh activity) and an increase in the unspecified cholinesterase fraction (BCh activity). In Table II the reversals of cholinesterase fraction patterns for the various diagnostic groups have been summarized. Of the reversals, 77% were found in the two traumatic groups of patients, whereas 91% of the cases showing no reversals were in the nontraumatic groups. Of the five nontraumatic cases with a tendency toward cholinesterase fraction reversals there were two patients with focal epilepsy and three patients with subarachnoid hemorrhage, hydrocephalus, and third ventricle tumor (with bilateral ventriculocisternostomies) respectively.

If the results of Tables I and II are compared, it is evident that patients with craniocerebral trauma showed striking differences from the rest of the patients in regard to cerebrospinal fluid analyses. These abnormalities recalled

the experimental concussion studies of Bornstein (3, 4).   Accordingly detailed clinical and E.E.G. studies were combined with cerebrospinal fluid analyses in following the course of these patients.

CHARACTERIZATION of CHOLINESTERASES in HUMAN CEREBROSPINAL FLUID

AVERAGE VALUES for VARIOUS DIAGNOSTIC GROUPS
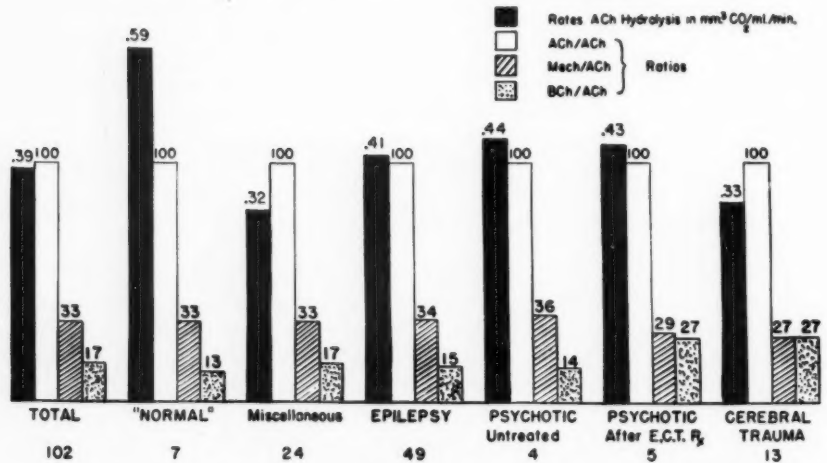
Fig. 1. *Correlation of cerebrospinal fluid cholinesterase values with clinical diagnoses in 102 patients.   (Diagnoses of craniocerebral trauma and psychotic cases are given in Tables III and IV respectively.   Diagnoses of miscellaneous group were: intracranial tumor, five; no CNS disease, four; cerebral thrombosis, three; headache, CNS syphilis, hydrocephalus, Arnold-Chiari malformation, adhesive arachnoiditis, and aneurysm, two each; histamine cephalalgia, trigeminal neuralgia, myasthenia gravis, Menière's disease, cerebral aplasia, idiopathic increased intracranial pressure, and multiple sclerosis, one each.).*

TABLE II

CEREBROSPINAL FLUID SAMPLES SHOWING REVERSAL OF NORMAL CHOLINESTERASE FRACTION PATTERNS

Sample type	Reversal		No reversal		Total
Craniocerebral trauma patients	12	(63 %)	7	(37 %)	19
E.C.T. treated psychiatric patients	5	(83 %)	1	(17 %)	6*
Untreated psychiatric patients	0		4	(100 %)	4*
Epileptics	2	(4 %)	47	(96 %)	49
Others	3	(9 %)	30	(91 %)	33
Total	22	(20 %)	89	(80%)	111

* *One patient examined before and after E.C.T.*

2. *Correlation of Cerebrospinal Fluid Analyses with Clinical and Electro-encephalographic Observations*

To illustrate our findings four of the cases of craniocerebral trauma are briefly summarized and discussed.

(1) *Patient 911 JK*, ♂, 37

Diagnosis.—Bilateral subdural hydromata, simple skull fracture of the right base and parietal bone, craniocerebral injury (Fig. 2).
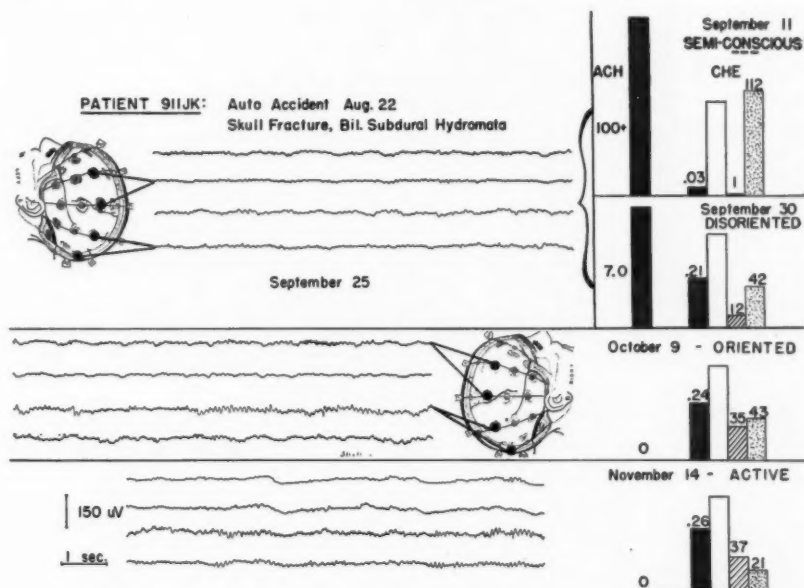
FIG. 2.   *Clinical, electroencephalographic, and cerebrospinal fluid acetylcholine and cholin-esterase findings on Patient 911 JK.   (For legend see Fig. 4).*

Case abstract.—Automobile accident, Aug. 22. Rendered immediately unconscious. Admitted 24 hr. later deeply comatose with right hemiparesis, bilaterally positive Babinski signs and rectal temperature of 107° F. Comminuted fracture of right base and parietal bone demonstrated by X-ray. Bilateral subtemporal craniotomies with drainage of bilateral sub-dural hydromata carried out at once. Remained semicomatose for the next 20 days. Pneumo-encephalogram on Sept. 11 demonstrated reaccumulation of subdural fluid. Following redrain-age patient responded but was disoriented, drowsy, and at times manic and hallucinating. He became oriented on Oct. 2, and was discharged on Oct. 10. He was followed for the next year during which time he returned to active work.

The first cerebrospinal fluid sample was obtained at the time of pneumo-encephalography (Sept. 11), 20 days after the accident while the patient was still semicomatose. It contained the relatively large amount of 100+ μgm. % of acetylcholine and showed a virtual absence of the specific cholinesterase fraction and a markedly reduced rate of acetylcholine hydrolysis (Figs. 2 and 3). An E.E.G. on Sept. 25 during the period of somnolence and disorientation

showed extremely low voltage records from all head leads with occasional random slow waves from the right centrotemporal region. The second cerebrospinal fluid sample, taken during the same clinical period (on Sept. 30), contained 7.0 μgm. % of acetylcholine and showed some improvement in cholinesterase activity with reappearance of the specific cholinesterase fraction. One week after the patient became rational an E.E.G. (Oct. 9) showed improvement with increased amplitude, return of alpha rhythm on the right and random slow waves from the left central region. A cerebrospinal fluid sample on the same day contained no acetylcholine and showed further return of the cholinesterase activity and fraction patterns toward normal. Re-examination a month after discharge (on Nov. 14) demonstrated still further improvement in the E.E.G. with return of alpha rhythm on the left and a decrease in slow wave activity. The electroencephalographic recovery was considered in general to be very good. The final cerebrospinal fluid analysis at this time showed absence of acetylcholine and a relatively normal cholinesterase fraction pattern together with further increase in cholinesterase activity (ACh hydrolysis).

The case of *911 JK* presented the most marked changes of any we observed, but it is typical of the findings in all the traumatic cases studied. There were, of course, other factors in this case such as an initial pyrexia of 107° F. and operative reintervention. The justification for assuming a correlation between E.E.G. and clinical state and the cerebrospinal fluid acetylcholine and cholinesterase values rests with the other cases in our study.

(2) *Patient 1118 NG, ♂, 19*

Diagnosis.—Right basilar skull fracture, right epidural hematoma, cerebral contusion, craniocerebral injury (Fig. 4).

Case abstract.—Struck by hit-and-run driver on Oct. 13 and rendered momentarily unconscious. During next three hours developed headache, irritability, vomiting, and drowsiness. On examination he was found to have right basilar skull fracture by X-ray and grossly bloody cerebrospinal fluid under increased pressure. Several days later decerebrate attacks developed. Transferred to Montreal Neurological Institute on Oct. 20, where examination showed stiff neck, positive Kernig's sign, left seventh nerve paresis, paresis of left arm, and bilaterally positive Babinski signs. Ventriculogram demonstrated a fluid collection over right temporal region. Right subtemporal craniotomy and evacuation of an epidural hematoma were carried out at once. Because of his failure to respond re-explorations were carried out one and three days later, but the patient remained in a semicomatose state for the next three months. By the end of January slight improvement was evident. This became definite in the next three months. He responded to commands, walked with help, and eventually could feed himself and call for necessary articles, although he remained unable to articulate. During this period he was very restless and difficult to control. He continued to improve, regaining ability to speak, and was discharged in September.

The first cerebrospinal fluid sample was drawn about one month after admission (on Nov. 18) while the patient was still in a semicomatose state. It contained acetylcholine in a concentration of 2.0 μgm. % with a reduction of cholinesterase activity and a reversal of the normal fraction patterns (Fig. 4). E.E.G. at this time showed depression of cortical activity with bursts of high amplitude slow waves, particularly from the right temporoparietal region. Three weeks later (on Dec. 9) a second cerebrospinal fluid analysis was made. It contained 1.5 μgm.% acetylcholine and showed a

PLATE I
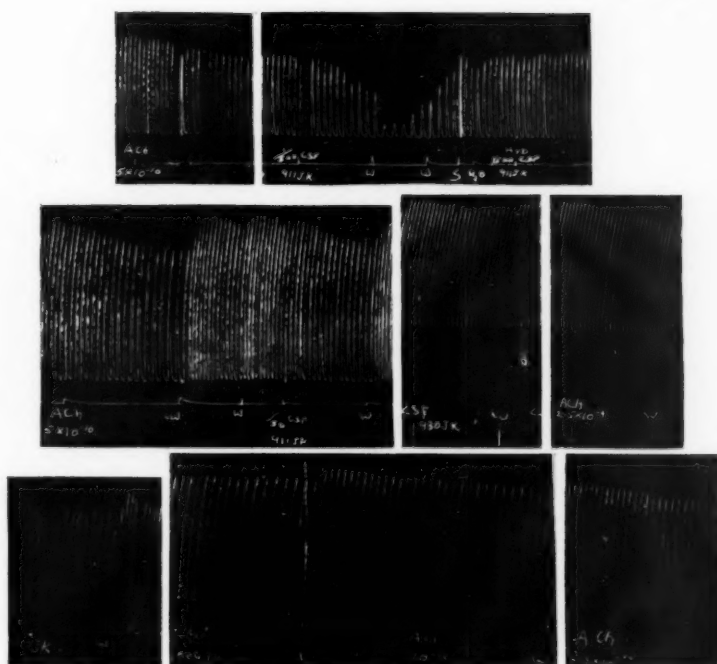
FIG. 3. *Kymograph records of cerebrospinal fluid acetylcholine assays on Patient 911 JK by the Venus heart method of assay (21).*

(Legend: *ACh* = *acetylcholine (In concentrations of* 2.5×10^{-9} = 10.0 *μgm.* %
5.0×10^{-10} = 2.0 *μgm.* %
2.5×10^{-10} = 1.0 *μgm.* %
10^{-10} = 0.4 *μgm.* %)

w = *wash*
911JK = *CSF sample of Sept. 11 (diluted 1 : 500 and 1 : 50 as noted)*
930JK = *CSF sample of Sept. 30*
1009JK = *CSF sample of Oct. 9)*
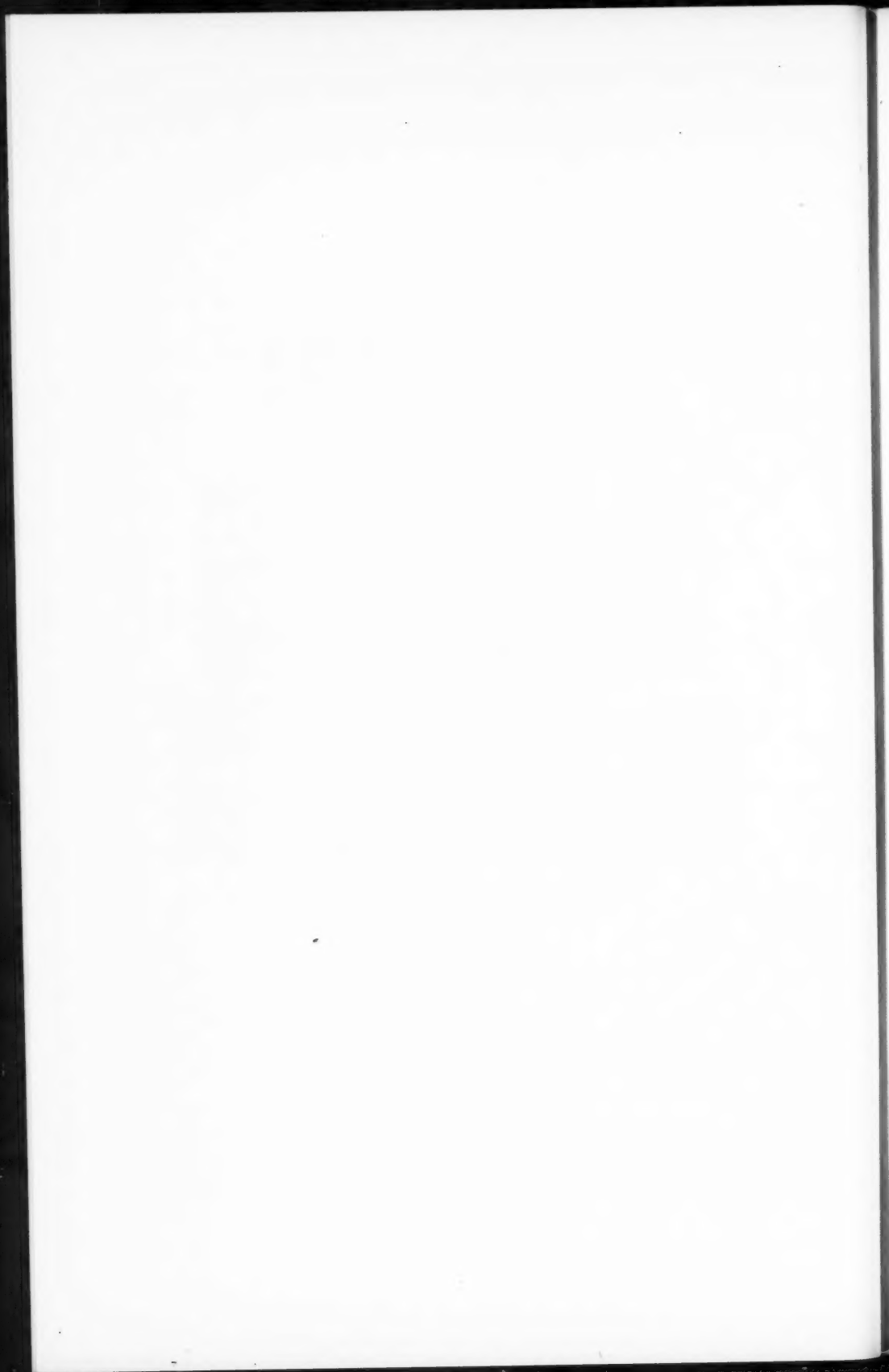
further decrease in cholinesterase activity and more marked reversal of the fraction pattern.   No change in clinical state had been observed, and an E.E.G. at this time showed some deterioration from the previous record with evidence of increasing severity of right temporoparietal damage.   A third cerebrospinal fluid analysis was made three months after admission (on Jan. 22) at which time clinical improvement was beginning.   In the cerebrospinal fluid 1.0 μgm. % of acetylcholine was still present, but cholinesterase activity and fraction patterns showed a tendency toward improvement.   The E.E.G. at this time was improved with reappearance of alpha rhythm on the left and a decrease in amplitude of the slow waves.   On May 10 after three months of gradual improvement a final cerebrospinal fluid analysis showed disappearance of acetylcholine and a definite return of cholinesterase values toward normal. E.E.G. at this time demonstrated continuing improvement, characterized by reappearance of alpha rhythm on the right and a disappearance of slow waves,


FIG. 4. *Clinical, electroencephalographic, and cerebrospinal fluid acetylcholine and cholinesterase findings on Patient 1118 NG.*

although some slow and sharp waves reversing over the right temporoparietal region suggested development of an epileptogenic focus there.   The interesting feature of this case is the prolonged period of semicoma without clinical or electroencephalographic improvement during which time the abnormalities in the cerebrospinal fluid acetylcholine and cholinesterase values persisted.

(3) *Patient 0109 GB*, ♂, 29

Diagnosis.—Left temporoparietal skull fracture, bilateral subdural hematomata, craniocerebral injury (Fig. 5).

FIG. 5. *Clinical, electroencephalographic, and cerebrospinal fluid acetylcholine and cholinesterase findings on Patient 0109 GB (Note: CSF analysis dated Jan. 13 should read Jan. 20).*

Case abstract.—On Dec. 18 patient was thrown from jeep landing on his head. He remained in a restless, semicomatose state for five days at which time he was transferred to the Montreal Neurological Institute. On admission patient was comatose, with a left seventh nerve paresis, left hemiparesis, and left hyperreflexia. A linear left temporoparietal skull fracture was demonstrated by X-ray. Bilateral subdural hematomata were tapped by twist-drill holes immediately after admission, following which patient regained consciousness but remained confused and disoriented. Operative drainage of subdural spaces carried out the following day. Remained confused until Jan. 12 after which he was oriented and rational. Discharged on Jan. 23 well.

An E.E.G. at the time of admission on Dec. 23, when the patient was still comatose, showed evidence of generalized brain damage with absence of normal alpha rhythm (Fig. 5). During the period when the patient was confused and disoriented, the first cerebrospinal fluid sample was drawn (Jan. 9). It contained no acetylcholine but showed the typical abnormalities of cholinesterase values. An E.E.G. (Jan. 13), when the patient had become rational, showed marked improvement except for some bursts of four to six per second slow and sharp waves. A second cerebrospinal fluid sample (on Jan. 20) three days before discharge showed normal cholinesterase values. In this case the initial cerebrospinal fluid sampling was somewhat late in the post-traumatic course. Recovery had apparently progressed beyond the point where acetylcholine could still be detected in the cerebrospinal fluid, although the residual cholinesterase changes remained.

(4) Patient 0216 VB, ♂, 30

Diagnosis.—Right frontoparietal skull fracture, cerebral contusion, craniocerebral injury; multiple fractures right facial bones, contusion right eye (Fig. 6).
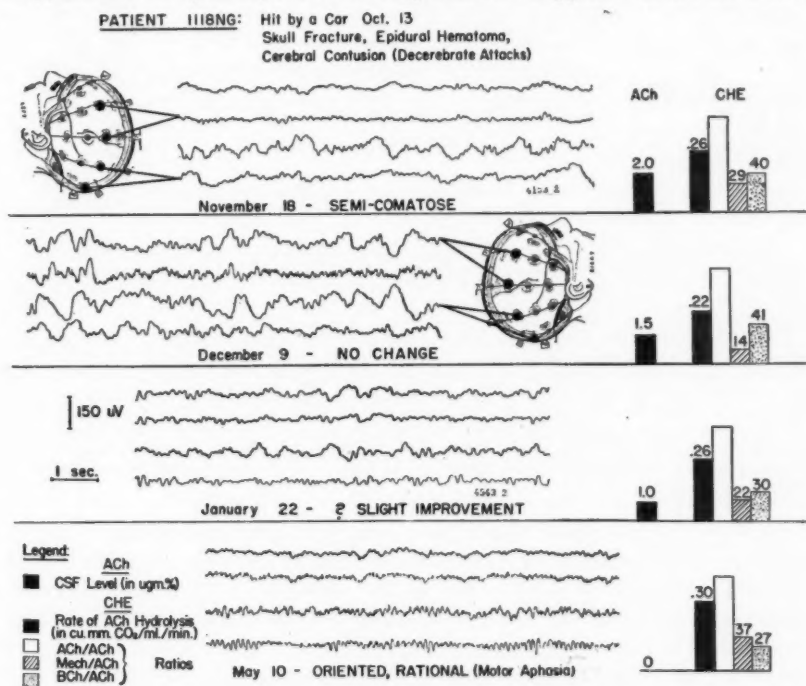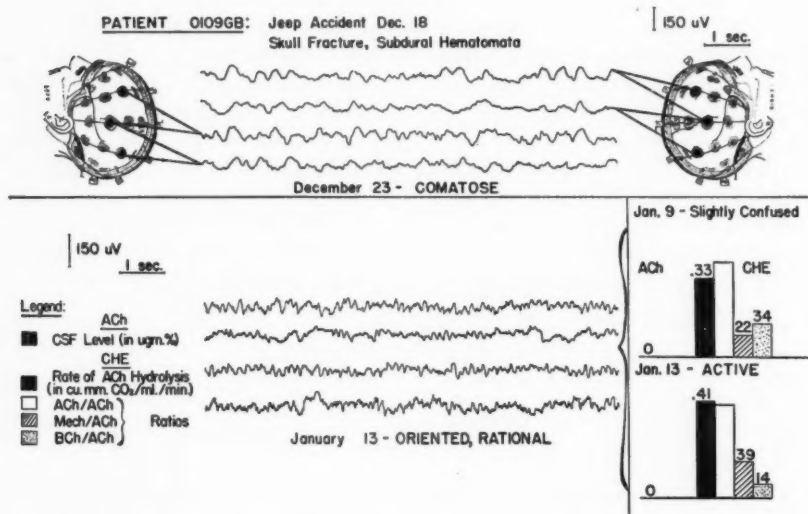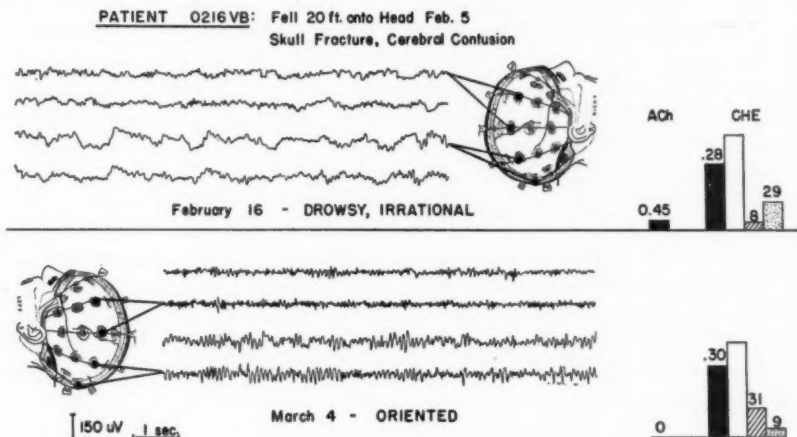

FIG. 6. *Clinical, electroencephalographic, and cerebrospinal fluid acetylcholine and cholinesterase findings on Patient 0216 VB (For legend see Fig. 5).*

Case abstract.—Four days before admission (Feb. 5) patient fell estimated 20 ft. onto head. Not rendered unconscious but subsequently became drowsy and irrational. On admission no neurological findings other than mental state of drowsiness and irrationality were elicited, but there was contusion of the right eye and evidence of multiple fractures of facial bones on the right. Right frontoparietal linear skull fracture demonstrated by X-ray. Pneumoencephalogram on Feb. 16 suggested possibility of space-occupying lesion on the right, but exploration of subdural and subarachnoid spaces was negative. Diagnosis of intracerebral hemorrhage was entertained. Gradual improvement took place, and he became clear and rational on Feb. 24. Discharged well on Mar. 5.

An E.E.G. on Feb. 16 showed very little rhythmic activity, high amplitude slow waves, and evidence of right frontoparietal damage (Fig. 6). Cerebrospinal fluid analysis at this time showed a small amount of acetylcholine and typical cholinesterase abnormalities. Ten days later (Mar. 4) at the time of discharge the E.E.G. was markedly improved with more regular alpha rhythm, decrease in slow wave activity, and higher amplitude of cortical activity. The cerebrospinal fluid sample taken at this time contained no acetylcholine and had normal cholinesterase values.

All 15 craniocerebral trauma cases studied are summarized in Table III. There is considerable variation from case to case, depending upon the time of sampling and the degree of trauma. As typified by the four cases cited (Figs. 2, 4, 5, 6) the essential features were the following: *first*, the presence initially of acetylcholine in the cerebrospinal fluid together with reduced activity and reversal of the normal fraction patterns of cerebrospinal fluid cholinesterases, so that the unspecified cholinesterase fraction predominated; *second*, the coinciding of these cerebrospinal fluid findings with depression of

TABLE III

CEREBROSPINAL FLUID ACETYLCHOLINE AND CHOLINESTERASE VALUES, ELECTROENCEPHALOGRAMS, AND CLINICAL STATES IN 15 CASES OF CRANIOCEREBRAL TRAUMA

Patient	Sex	Age	Diagnosis	Date	Cells per ml. RBC	WBC	Pandy	Protein in mgm.%	ACh in μgm.%	ChE rate**	MeCh/ACh	BCh/ACh	E.E.G. record	Clinical state
909 JMcI	♂	32	Post-traumatic headache	9-9	10	2	+	35.3	0/4	0.25	43	35	Normal	Normal
911 JK	♂	37	Bilateral subdural hydromata; basal skull fracture	9-11	127	52	2+	45.1	100+	0.03	1	112	(Severely depressed)	Semicoma
				9-30	10	1	2+	79.5	7.0	0.21	12	42		Disoriented
				10-9	120	0	3+	97.2	0/0.3	0.24	35	43	Improved	Oriented
				11-14	2	0	—	72.5	0/1	0.26	37	21	Good recovery	Active
				9-19	7	7		19.2	0/0.4	0.25	32	15		Active
919 DB	♂	14 mos.	Bilateral subdural hydromata											Active
922 AB	♂	29	Post-traumatic headache	9-22	4	2	+	24.7	0/0.3	0.27	39	19	Rhythmic instability	Active
923 GM	♂	20	Cerebral contusion	9-23	52	0	+	29.7	—	0.26	26	20	—	Recovering
1118 NG	♂	20	Right epidural hematoma; right basilar skull fracture; cerebral contusion	11-18	34	8	+	37.1	2.0	0.26	29	40	Depressed	Semicoma
				12-9	93	21	+	57.4	1.5	0.22	14	41	Deteriorating	Semicoma
				1-22	1	0	0	—	1.0	0.26	22	30	Slight improvement	Semicoma, improving
1119 JS	♂	51	Right subdural hematoma	5-10	16	2	0	—	0/0.2	0.30	37	27	Marked recovery	Oriented
				11-20		2	+	64.2	0/1	0.37	26	25	Improved	Recovering
1120 AF	♂	24	Right temporal skull fracture	11-20	20	0	—	18.7	0/1	0.41	27	19	—	Recovering
0109 GB	♂	29	Left temporoparietal skull fracture; bilateral subdural hematoma	1-9	9	10	±	31.6	0/0.3	0.33	22	34	(Much improved)	Confused
				1-20	163	77	±	30.5	0/1	0.41	39	14		Recovering
0109 JLAP	♂	18	Cerebral contusion	1-9	14	14	+	49.1	0/0.3	0.64	34	22	Normal	Active
0110 AF	♂	58	Right temporoparietal skull fracture; right epidural hematoma; cerebral contusion	1-10	15	0	±	32.5	0/0.3	0.34	39	46	—	Semicoma
0216 VB	♂	30	Right frontoparietal skull fracture; cerebral contusion	2-16	—	—	+	44.3	0.45	0.28	8	29	Depressed	Drowsy
				3-4	15	36	0	61.0	0/0.1	0.30	31	9	Much improved	Oriented
0226 ED	♂	42	Left parieto-occipital multiple compound depressed skull fractures; left epidural hematoma; cerebral contusion	2-25	—	—	2+	97.2	0.2	0.60	20	27	Low amplitude	Manic
V1205 KJ***	♂	3 mos.	Hydrocephalus, communicating, post-traumatic	12-5	—	—	4+	527	0	2.93	4	50		
0203 CB***	♂	45	Craniocerebral injury	2-3	—	—	4+	1106	0	1.05	10	50		
911 JK***	♂	37	(See above)	9-11	—	—	4+	180	0	2.23	18	47		

* Acetylcholine levels that are negative are expressed as 0/threshold of test object in μgm.%.

** Cholinesterase rate in cu. mm. CO_2/ml. CSF/min.

*** Subdural fluid samples.

the E.E.G. and the clinical state; *third*, parallel improvement of E.E.G. and clinical state with disappearance of acetylcholine from the cerebrospinal fluid and return toward normal of cerebrospinal fluid cholinesterase values (the cholinesterases apparently being more sensitive indicators in this regard); and *fourth*, an apparent rough correlation of acetylcholine levels in the cerebrospinal fluid with the extent of clinical and E.E.G. abnormalities, so that with high acetylcholine levels depression was observed, while at low acetylcholine levels excitatory phenomena occurred.   The observations correspond in general to the more precise experimental studies of Bornstein (3).   In some cases other factors, in particular operative intervention, must be taken into account.   Contamination of cerebrospinal fluids with subdural fluid, as seen in the last two cases of Table III (*V1205 KJ* and *0203 CB*), can be readily differentiated (22).   In our other reports, factors of age, sex, cerebrospinal fluid cellular reaction, cerebrospinal fluid protein level, and medication of the patients have been shown to be of little or no significance for the results of cerebrospinal fluid acetylcholine and cholinesterase determinations.   With the exception of cases of epilepsy and craniocerebral trauma clinical diagnoses have not had any bearing on results. The finding of acetylcholine in the cerebrospinal fluid in the presence of considerable amounts of cholinesterases has been shown to be possible because of the minimal or absent cholinesterase activity at such suboptimal substrate concentrations (8, 22, 23).

3. Cerebrospinal Fluid Acetylcholine and Cholinesterase Studies in Nine Psychiatric Patients

In the nine psychiatric patients available for study only cerebrospinal fluid acetylcholine and cholinesterase determinations were possible.   Four of these patients received no E.C.T. treatments prior to sampling.   One of these (patient *0121 EF*) was resampled after five E.C.T. treatments.   The other five patients were sampled only after several E.C.T. treatments had been given.*   The diagnosis and results of cerebrospinal fluid studies on these patients are summarized in Table IV.

Cerebrospinal fluid samples from the four untreated patients contained no acetylcholine and had normal cholinesterase values.   In contrast the E.C.T. treated patients, with a single exception (patient *0120 HO'R*), all showed changes in cerebrospinal fluid cholinesterase fraction patterns similar to those previously described for cases of craniocerebral trauma, namely, an increase in the unspecified cholinesterase fraction (BCh activity) and a decrease in the specific cholinesterase fraction (MeCh activity).   In only two of the E.C.T. treated patients was acetylcholine found in the cerebrospinal fluid samples. Patient *0121 EF* is most interesting because pre- and post-treatment samples

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* *All electric shock convulsant therapy (E.C.T.) was given at the Allan Memorial Institute of the Royal Victoria Hospital.   The patients were routinely pretreated to drowsiness with intravenous sodium amytal.   Electric shocks were administered from a standard Offner or Rahm machine through two electrodes, placed on vertex and temple respectively.   In all these cases a convulsant dose of 700 ma. at a pulse interval of 0.7 msec. was applied for a treatment duration of two seconds.*

TABLE IV

CEREBROSPINAL FLUID ACETYLCHOLINE AND CHOLINESTERASE VALUES IN NINE
PSYCHIATRIC PATIENTS

Patient	Sex	Age	Diagnosis	No. of E.C.T.	ACh in μgm.%*	ChE Rate**	ChE ratios	
							MeCh/ ACh	BCh/ ACh
1209 TD	♂	19	Anxiety state	0	0/1	0.42	36	12
1230 EM	♂	37	Infectious psychosis	0	0/0.02	0.42	37	9
0128 AG	♀	43	Anxiety state	0	0/0.3	0.60	34	18
0121 EF	♀	48	Manic-depressive	{ 0	0/0.1	0.32	36	16 }
				5	0.2	0.32	29	30 }
0119 RS	♀	37	Manic-depressive	6	1.0	0.59	18	32
0119 DM	♀	37	Reactive depression	6	0/1	0.25	28	30
0120 HO'R	♀	56	Involutional melancholia	7	0/1	0.62	35	18
0120 IG	♀	32	Reactive depression	3	0/1	0.48	33	29
0130 EC	♀	36	Manic-depressive	3	0/1	0.30	36	30

* *Acetylcholine levels that are negative are expressed as 0/threshold of test object in μgm. %.*
** *Cholinesterase rate in cu.mm. CO_2/ml. CSF/min.*

were obtained. The appearance of acetylcholine in the cerebrospinal fluid and the reversal of cholinesterase fraction patterns after five E.C.T. treatments were quite striking in this patient.

The number of cases and the number of samples on each case are small but some conclusions seem warranted. The most marked cerebrospinal fluid abnormalities seemed to occur in cases with the most E.C.T. treatments. Because of the small quantities of acetylcholine found it is possible that some low acetylcholine values were missed. No explanation for the exception of patient *0120 HO'R* to the general trend of cholinesterase values in the other five patients could be found. It is interesting that this patient was the only one of the six to show no response to treatment.

Because of the findings presented in Table IV, together with the findings in accidental craniocerebral trauma (Table III) and cases of spontaneous epileptic seizures (22), we regard the cerebrospinal fluid abnormalities in E.C.T. treated cases as due to a kind of cerebral trauma produced by the electric shock. The less márked cerebrospinal fluid abnormalities in E.C.T. cases would be consistent with the milder degree of trauma delivered. In dogs given convulsant doses of electric shock Bornstein (4) found acetylcholine in the cerebrospinal fluids of several animals. Bornstein and Stern (5), Brecht and Kummer (6), and Himwich (11) have reported finding acetyl-choline in the cerebrospinal fluids of patients following electric shock therapy. None have reported on the cerebrospinal fluid cholinesterases. Abnormalities in the E.E.G. (slow wave activity) (13) and atrophic gyri beneath the sites of electrode application (19) have been found after repeated E.C.T. treatments. These observations support our findings in the nine psychiatric patients studied.

Discussion

The study of patients is beset with difficulties that can often be circumvented in animal experiments. Bornstein (3) observed during perfusion of the cortex of the unanesthetized dog and cat a correlation between acetylcholine levels in the cerebrospinal fluid and the E.E.G. pattern and behavior of the animals. We have observed this in the human cases reported here. With high acetylcholine levels (over 1.0 to 2.0 μgm. %) disturbances of consciousness up to semicoma or coma together with depression of electroencephalographic activity were seen by both Bornstein (3) and us. As the level of acetylcholine decreases, patients and animals went through a period of restlessness and confusion before becoming normal. This was associated with increased activity (particularly slow and sharp waves) in the E.E.G. The cerebrospinal fluid cholinesterase changes, observed in our patients, seem to be even more sensitive indicators of the post-traumatic state than either the cerebrospinal fluid acetylcholine level or the E.E.G. The persistence of cholinesterase changes for long periods of time and their reversion to normal values coincident with clinical improvement are significant.

It is not clear what the mechanisms are for the findings reported here. Acetylcholine may be liberated because of trauma to cerebral tissue (15) or during mass neuronal discharges following trauma (25) or both. Bornstein (3) has shown that acetylcholine in the concentrations found can produce the observed effects on the E.E.G. and clinical state. In our study of epileptic patients 77% had cerebrospinal fluid samples positive for acetylcholine in concentrations lower than those seen in traumatic cases (8, 23). It has been suggested that the liberation of acetylcholine into these cerebrospinal fluids was due to the repeated trauma to the cortex of epileptic seizures. If this were so, changes in cerebrospinal fluid cholinesterases might also be expected to be present. This is not the case (22), which suggests that the appearance of acetylcholine in cerebrospinal fluids of epileptics involves a different mechanism from that seen in craniocerebral trauma.

Where the cerebrospinal fluid cholinesterase abnormalities also fit into the picture is difficult to say. We have previously suggested that the specific cholinesterase fraction is derived from nervous tissue and that the unspecified fraction is contributed by an extra-neural source (22). The increased permeability of the blood–brain barrier following trauma could account for the increase in unspecified cholinesterase. The report of Kabat *et al.* (14) is in accord with this idea. Since Cohn *et al.* (7) have shown unspecified cholinesterase to be carried in the alpha$_2$ globulin plasma protein fraction, it should be possible to apply the methods of Kabat *et al.* (14) to the further elucidation of this problem. The changes in the specific cholinesterase fraction may be due to relative inactivation of this enzyme fraction by increased destruction, decreased production, temporary inhibition, dilution with anenzymatic fluid, or any combinations thereof. It is conceivable that in certain cases craniocerebral trauma might impair the capacity of specific cholinesterase in cerebral

tissue to cope with the high concentrations of acetylcholine liberated, so that the clinical course of the patient could be considerably altered (22). We feel that changes in the cerebrospinal fluid reflect changes in cerebral tissues and that attention should now be directed to an investigation of these tissues.

Further studies of the problem may result in new treatments of the post-traumatic patient. The use of atropine to counteract the effects on the E.E.G. and clinical state of abnormally high cerebrospinal fluid concentrations of acetylcholine has been suggested by Bornstein (3). Darrow *et al.* (9), Grob *et al.* (10), and Wescoe *et al.* (26) have reported studies consistent with this idea. It is also of interest that Mendel and Hawkins (16) and Hyde *et al.* (12) have used cholinesterase experimentally to alter neuronal activity. Red blood cells, rich in specific cholinesterase, are still a relatively unused by-product of plasma production, which could be made available for trial in craniocerebral trauma. We have not yet had sufficient opportunity to investigate these and other promising therapeutic approaches. The possibilities for furthering our understanding of the pathological neurophysiological processes in craniocerebral trauma and of the biochemistry of neuronal activity are many.*

Acknowledgments

References

1. (a) AMMON, R. Arch. ges. Physiol. (Pflügers), 233 : 486. 1933.
   (b) AUGUSTINSSON, K-B. Acta Physiol. Scand. 15 (Suppl. 52) : 1. 1948.
2. BABKIN, B. P., SCHACHTER, M., DWORKIN, S., and ROSENBERG, G. M. Rev. can. biol. 1 : 761. 1942.
3. BORNSTEIN, M. B. J. Neurophysiol. 9 : 349. 1946.
4. BORNSTEIN, M. B. Unpublished data. 1948.
5. BORNSTEIN, M. B. and STERN, K. Unpublished data. 1948.
6. BRECHT, K. and KUMMER, H. Klin. Wochschr. 22 : 741. 1943.
7. COHN, E. J., STRONG, L. E., HUGHES, W. L., MULFORD, D. L., ASHWORTH, J. M., MELIN, M., and TAYLOR, H. L. J. Am. Chem. Soc. 68 : 459. 1946.
8. CONE, W. V., TOWER, D. B., and MCEACHERN, D. Trans. Am. Neurol. Assoc. 73 : 59. 1948.
9. DARROW, C. W., PATHMAN, J. H., and KRONENBERG, G. Federation Proc. 4 : 16. 1945.
10. GROB, D., HARVEY, A. M., LANGWORTHY, O. R., and LILIENTHAL, J. A. Bull. Johns Hopkins Hosp. 81 : 257. 1947.
11. HIMWICH, H. E. J. Am. Med. Assoc. 136 : 870. 1948.
12. HYDE, J. E., BECKETT, S., and GELLHORN, E. Federation Proc. 6 : 133. 1946.
13. JASPER, H. H. Personal communication. 1948.
14. KABAT, E. A., GLUSSMAN, M., and KNAUB, V. Am. J. Med. 4 : 653. 1948.
15. LORENTE DE NÓ, R. Am. J. Physiol. 121 : 331. 1938.

* *Since completion of this paper the extensive review on cholinesterases by Augustinsson (1b) has appeared. It contains many results and ideas germane to the present study.*

16. MENDEL, B. and HAWKINS, R. D.   J. Neurophysiol. 6 : 431.   1943.
17. NACHMANSOHN, D. and ROTHENBERG, M. A.   J. Biol. Chem. 158 : 653.   1945.
18. ODOM, G., RUSSEL, C. K., and McEACHERN, D.   Brain, 66 : 1.   1943.
19. PENFIELD, W.   Personal communication.   1948.
20. TOWER, D. B.   Can. J. Research, E, 27 : 20.   1949.
21. TOWER, D. B. and McEACHERN, D.   Can. J. Research, E, 26 : 183.   1948.
22. TOWER, D. B. and McEACHERN, D.   Can. J. Research, E, 27 : 132.   1949.
23. TOWER, D. B. and McEACHERN, D.   Can. J. Research, E, 27 : 120.   1949.
24. WAIT, R. B.   Biol. Bull. 85 : 79.   1943.
25. WALKER, A. E., KOLLROSS, J. J., and CASE, J. J.   J. Neurosurg. 1 : 103.   1944.
26. WESCOE, W. C., GREEN, R. E., McNAMARA, B. P., and KROP, S.   J. Pharmacol. Exptl.
       Therap. 92 : 63.   1948.

ACETYLCHOLINE AND NEURONAL ACTIVITY

II. ACETYLCHOLINE AND CHOLINESTERASE ACTIVITY IN THE CEREBROSPINAL FLUIDS OF PATIENTS WITH EPILEPSY[1]

By Donald B. Tower and Donald McEachern

Abstract

Cerebrospinal fluid acetylcholine assays were done on 119 samples from 109 neurological and neurosurgical patients. Of these 56 were epileptic and 53 non-epileptic patients. Acetylcholine was present in the cerebrospinal fluids of eight (15%) of nonepileptics, six of whom were cases of craniocerebral trauma (previously shown to give positive assays). In contrast 44 (77%) of epileptic patients had positive cerebrospinal fluid acetylcholine assays in amounts of 0.02 to 5.0 μgm. % acetylcholine (average 1.0 μgm. %). The presence of acetylcholine in cerebrospinal fluids of epileptics was apparently related to the frequency of seizures, the occurrence of seizures, and the extent of electroencephalographic abnormalities in these patients. Factors of cerebrospinal fluid cholinesterase values, medications, thresholds of assay methods, and types of epilepsy (with the exception of focal seizures due to intracranial tumor) seemed to be of no significance for these results. This study does not indicate whether the acetylcholine recovered in epileptic cases was directly related to the occurrence of seizures or merely a coincidental finding. The presence of acetylcholine in cerebrospinal fluids in association with epileptic seizures is considered to be significant in the light of previous studies discussed.

Introduction

There is much evidence to suggest a role for acetylcholine in the production of seizures. The present study is part of an attempt to define the biochemical mechanisms that underlie the epileptic discharge. The earlier literature has been well reviewed by Dale (18), Loewi (32), Feldberg (20), and Nachmansohn (38, 39), so that only pertinent studies will be considered here. Sjöstrand (47) was the first to observe an increase in cortical activity (by electroencephalography) with local application of small amounts of acetylcholine, eserine, and the like. Subsequent studies by Miller (36), Miller et al. (37), Williams and Russell (55), Brenner and Merritt (11), Merritt and Brenner (35), Chatfield and Dempsey (14), Forster et al. (24), Forster and McCarter (25), Bornstein (6), Hyde et al. (29), Grob et al. (27), and Freedman et al. (26) confirmed these findings. The effect of acetylcholine on cortical electrical activity was found to be similar to that of direct electrical stimulation or of indirect stimulation by nerve impulses and has been termed "epileptiform" in character (30).

These observations led Forster (23) to call acetylcholine unique among true convulsants in that it is normally present in the brain and can cause seizure discharges. Several specific reports may be cited in support of this statement. Bornstein (6) demonstrated the production of "epileptiform" activity in the electroencephalogram during perfusion of the unanesthetized animal cortex

with 1.0 to 2.0 μgm. % of acetylcholine. Beutner (5) found that acetyl-
choline produced electromotive effects at phase boundaries in far smaller
concentrations than any other physiological substance. Mendel and Hawkins
(34) were able to inhibit nerve activity *in vivo* by injection of cholinesterases,
and subsequently Hyde *et al.* (29) inhibited or delayed electrically induced
convulsions by pretreatment of the animal cortex with cholinesterases. Grob
et al. (27) and Freedman *et al.* (26) observed epileptiform activity in the electro-
encephalogram following administration to animals and man of the cholin-
esterase inhibitor, diisopropylfluorophosphate (D.F.P.). Pope *et al.* (45)
found that cholinesterase activity of experimentally produced focal epilepto-
genic lesions was increased compared to surrounding and distant "normal"
cortical tissues. On the basis of Nachmansohn's work (38, 39), Pope *et al.*
interpreted their results to indicate that acetylcholine metabolism might be
increased in the epileptogenic tissue. Elliott (19) could not demonstrate any
abnormality of respiratory metabolism in epileptogenic cortical tissues as
compared with normal cortical tissues from animals and man.

We have sought to obtain further information on the possible role of acetyl-
choline in the mechanism of seizures. Our method of attack was based on
Bornstein's work (6) in which cerebrospinal fluids were found to contain
acetylcholine following experimental concussion. The recovery of acetyl-
choline from the cerebrospinal fluids of animals was first reported by Feldberg
and Schriever (22), and subsequently by Adam *et al.* (1), Chang *et al.* (12, 13),
Chute *et al.* (15), and Berger (4). Its recovery from cerebrospinal fluids of
man and animals was reported by Parrot and Lefebvre (43), Brecht and
Kummer (10), Bornstein (7), Bornstein and Stern (8) following electrical
stimulation of the cortex, and by Himwich (28) following metrazol convul-
sions.* In only three instances has the association of acetylcholine in the
cerebrospinal fluid with human epilepsy been reported (41, 9, 7).

Materials and Methods

Determinations were made on 119 cerebrospinal fluid samples from 109
neurological and neurosurgical patients for acetylcholine, cholinesterases, and
routine constituents. Of the samples, 80% were obtained during pneumo-
encephalography, the remainder during ventriculography (4 %) and lumbar
puncture (16 %). Cell counts, Pandy tests, and protein determinations (16)
were done on all samples. Cerebrospinal fluid cholinesterase activities and
fraction characterizations were done by our modification of the Warburg
method of Ammon (2), Odom *et al.* (42), and Nachmansohn and Rothenberg
(40) and have been reported in a previous paper (51). Cerebrospinal fluid
samples for acetylcholine levels were preserved by a modification of the method
of Babkin *et al.* (3, 48) and were assayed by our modification of the method of

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* *The latter observation is of interest in view of the use of intravenous metrazol to activate
epileptic foci (17, 31).*

Wait (53) on the isolated ventricle of the mollusc, *Venus mercenaria* (50).
Electroencephalograms were taken on conventional four-channel ink-writing
recorders in cooperation with the Department of Electroencephalography.

Results

1. *Cerebrospinal Fluid Acetylcholine and Cholinesterase Values*

Of the 109 patients studied 56 were epileptics and 53 nonepileptics.*   In
all cases the history of or the observation of clinical seizures were the criteria
for placing patients in the epileptic group.   One epileptic was not examined for
cerebrospinal fluid acetylcholine.   One nonepileptic has been counted twice
because of a change in therapeutic status.   Because of the occurrence of a
single seizure during pneumoencephalography in two nonepileptic patients, it
seemed advisable to classify them in both groups.   The totals, therefore, in
most figures and tables in this paper will read 57 epileptics and 54 nonepileptics.

Results of cerebrospinal fluid acetylcholine assays in these two groups of
patients are summarized in Fig. 1.   It can be seen that 44 (77%) of the
epileptic patients were found to have acetylcholine in the cerebrospinal fluid
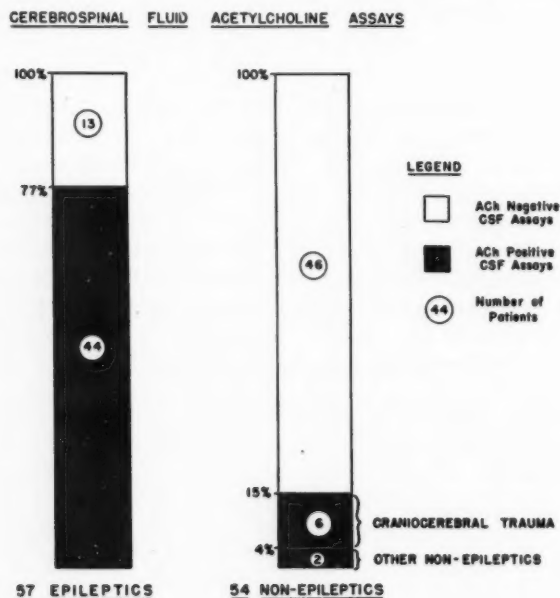
FIG. 1.   *Results of acetylcholine assays on cerebrospinal fluid samples from epileptic and
nonepileptic patients.*

* *The nonepileptic group comprised the following: craniocerebral trauma, 13; psychoneurosis
or psychosis, 10; intracranial tumor, five; headache (cause unknown), four; no CNS disease, four;
cerebral thrombosis, three; CNS syphilis, adhesive arachnoiditis, and Arnold-Chiari malformation,
two each; and Menière's disease, myasthenia gravis, trigeminal neuralgia, multiple sclerosis,
cerebral aplasia, hydrocephalus, cerebral aneurysm, and undiagnosed CNS disease, one each.*

at the time of sampling.   In contrast only eight (15%) of the nonepileptics
gave positive assays.   Of these latter eight patients, six fell into the group with
diagnoses of craniocerebral trauma, in whom positive acetylcholine assays
could be expected (52).   Thus only two nonepileptics (4% of the total)
remained is unexplained acetylcholine positives.   The clinical diagnoses in
these two cases were "No Central Nervous System Disease" and "Arnold-
Chiari Malformation", respectively.

The amounts of acetylcholine found in the cerebrospinal fluids of the 44
positive epileptics ranged from 0.02 to 5.0 μgm. % with an average value of
1.0 μgm. %.   In Table I the ranges of values have been summarized.

TABLE I

RANGE OF VALUES OF ACETYLCHOLINE FOUND IN
CEREBROSPINAL FLUID SAMPLES OF EPILEPTIC PATIENTS

Range in μgm. %	No. of samples	%
0.01 – 0.25	11	24
0.26 – 0.99	17	37
1.0 – 1.99	10	22
2.0 – 5.0	8	17
Total	46	100

Average value = 1.0 μgm. %

Almost two-thirds of the cerebrospinal fluid samples contained less than
1.0 μgm. % of acetylcholine.   This finding is in distinct contrast to our cases
of craniocerebral trauma in whom the average was over 2.0 μgm. %.   In
animals subjected to experimental concussion Bornstein (6) found cerebrospinal
fluid acetylcholine levels from 2.7 to 9.0 μgm. %.   Brecht (9) found up to
0.1 μgm. % of acetylcholine in cerebrospinal fluids of his few epileptic cases.
Attempts to correlate our cerebrospinal fluid acetylcholine levels with the
severity of the epileptic process proved inconclusive.   Representative *Venus*
heart kymograph tracings of positive and negative acetylcholine assays on
cerebrospinal fluid samples from the epileptic group of patients are illustrated
in Figs. 2 and 3 respectively.

 Cerebrospinal fluid cholinesterase determinations were carried out on a
total of 102 patients, including 49 of the 57 epileptics.   These findings are
discussed in detail in another paper (51).   It was found that the cholinesterase
values (activity) and fraction patterns were not significantly different from
those of the nonepileptic group, except for the changes previously reported
in cases of craniocerebral trauma (51; 52, Fig. 1).   Analysis of the epileptic
group failed to demonstrate any significant correlations between cholinesterase
values and types of epilepsy, seizure frequency, type of sample, presence or

absence of acetylcholine, or acetylcholine level (49).   The apparent paradox of the finding of acetylcholine in cerebrospinal fluids in the presence of significant amounts of cholinesterases is due to the low substrate concentrations.   The acetylcholine concentrations found (Table I) were about 0.001 % of the optimal concentrations normally used to measure cholinesterase activity. At such suboptimal concentrations of substrate the cerebrospinal fluid cholinesterase activity is practically nil (51, Fig. 5).

2. *Analysis of Results in Terms of Factors not Strictly Peculiar to Epilepsy*

The results of cerebrospinal fluid acetylcholine assays in both the epileptic and nonepileptic groups of patients were first analyzed in terms of factors common to both groups.   The factors of age and sex, of presampling medication with various sedatives, and of thresholds of the acetylcholine assay method failed to show any significant relationships to the results of acetylcholine assays on these samples.   The duration of the epileptic disease process also proved to be of no significance to the results.

Analysis of the possible effect of anticonvulsant medication given to epileptic patients on cerebrospinal fluid acetylcholine assays is given in Table II. The majority of our epileptic patients are taken off medication upon admission in order to obtain satisfactory electroencephalographic records.   Because of this fact the figures for medications prior to admission (*A*, Table II) are probably not significant.   During admission (*B*, Table II) the percentages of acetylcholine positive and negative assays for the 18 patients receiving medications compare well both with the percentages for the 18 patients who were not on medication at any time and also with the percentages for the 40 patients who were not on medication subsequent to admission.   Anticonvulsant medication, therefore, did not seem to have any significant effect on the results of cerebrospinal fluid assays for acetylcholine in these patients.

FIG. 2.   *Kymograph records of positive acetylcholine assays by the Venus heart method* (50) *on cerebrospinal fluid samples from epileptic patients.*

(*Legend: ACh*   = *acetylcholine in concentrations of 0.025   = 1.0 µgm.%*
                                                  0.00875 = 0.35 µgm.%
                                                  0.001   = 0.04 µgm.%
                                                  10^{-10} = 0.4 *µgm.%*

         w   = *wash*
         h or hyd   = *hydrolyzed sample*
         0129PP   = *CSF sample on patient with cerebral seizures, unlocalized*
         1128PMacC = *CSF sample on patient with focal left parieto-occipital seizures due to cystocercosis*
         1218CG   = *CSF sample on patient with focal right temporal seizures (cause undetermined)*
         1217GN   = *CSF sample on patient with focal left temporal seizures (cause undetermined)*
         916RMacD   = *CSF sample on patient with focal right temporoparietal seizures due to calcified ependymoma)*

PLATE I
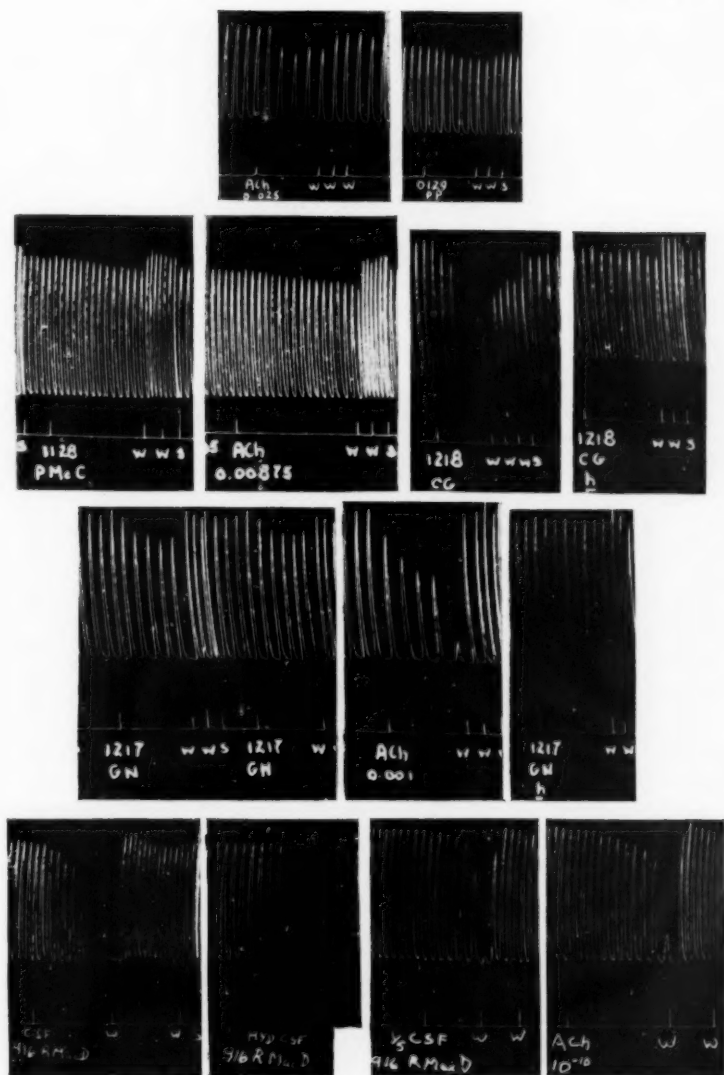
PLATE II
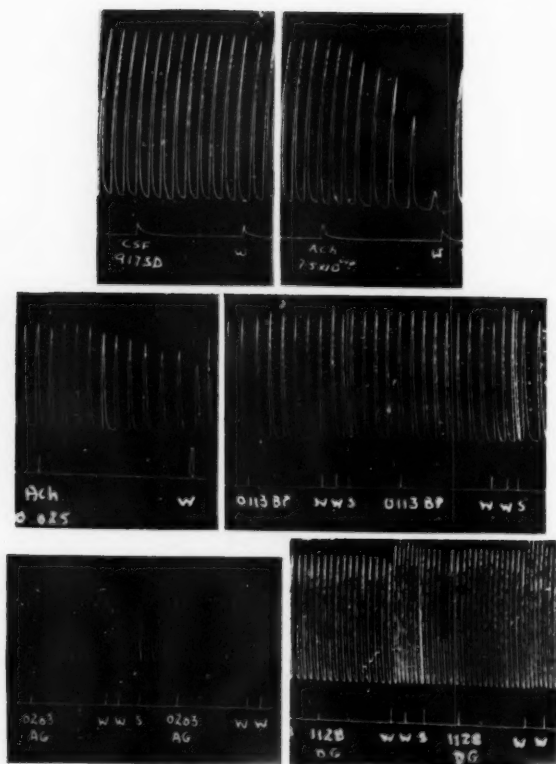
FIG. 3.   *Kymograph records of negative acetylcholine assays by the Venus heart method (50)*
on cerebrospinal fluid samples from epileptic patients.

(*Legend: ACh*   = *acetylcholine in concentrations of* 7.5 × 10⁻¹⁰ = 3.0 µgm.%
                                                     0.025   = 1.0 µgm.%

       w   = *wash*

       917SD   = *CSF sample on patient with focal left sensorimotor seizures due to*
                                      parasagittal meningeal fibroblastoma

       0113BP   = *CSF sample on patient with cerebral seizures, unlocalized*

       0203AG   = *CSF sample on patient with focal cerebral seizures (localization in*
                                      doubt) with tumor (suspected)

       1128DG   = *CSF sample on patient with focal insular seizures (lateralization and*
                                      cause undetermined))

TABLE II

EFFECT OF ANTICONVULSANT MEDICATION ON RESULTS OF CEREBROSPINAL FLUID ACETYLCHOLINE ASSAYS IN EPILEPTIC PATIENTS

Medication	Total samples	ACh positive		ACh negative	
A. *Preadmission*					
None	20	14	(70 %)	6	(30 %)
Phenobarbital	7	3	(44 %)	4	(56 %)
Dilantin	8	7	(88 %)	1	(12 %)
Phenobarbital and dilantin	11	11	(100 %)	0	
Others*	12	10	(83 %)	2	(17 %)
Total	58	45	(77 %)	13	(23 %)
B. *During admission*					
None	40	30	(75 %)	10	(25 %)
Phenobarbital	7	5	(71 %)	2	(29 %)
Dilantin	2	1	(50 %)	1	(50 %)
Phenobarbital and dilantin	6	6	(100 %)	0	
Others*	3	3	(100 %)	0	
Total	58	45	(77 %)	13	(23 %)
None (before or during admission)	18	12	(67 %)	6	(33 %)

* *Other medications were tridione, bromides, mebaral, gardenal, and sodium amytal.*

3. *Analysis of Results in Terms of Factors Peculiar to Epilepsy*

(A) *Types of Epilepsy*

The relationships of cerebrospinal fluid acetylcholine assays to clinical types of epilepsy given in Fig. 4 were based on Penfield's (44) classification of the epilepsies. From Fig. 4 it can be seen that the type of epilepsy was without significance except in cases of focal seizures due to intracranial tumor. In this latter group the seven negatives comprised over half the total of 13 acetylcholine negative assay cases encountered. Analyses of the types of tumors and their localizations were of no help in explaining this finding.†

(B) *Frequency of Seizures*

From case histories, three frequency groups for seizures prior to admission were chosen: *weekly*, one or more seizures per week; *monthly*, less than one seizure per week but one or more per month; and *yearly*, less than one

† *Tumor types were: ependymoma, one*; hemangioma, two*; astrocytoma (unclassified), one; astrocytoma diffusum, two*; glioma (type undetermined), one; meningeal fibroblastoma, two; tumor (suspected), two*. Localizations were: frontal, three*; precentral, two; temporal, two**; insular, one*; olfactory groove, one; not recorded, two. (Each * represents one acetylcholine positive assay).*

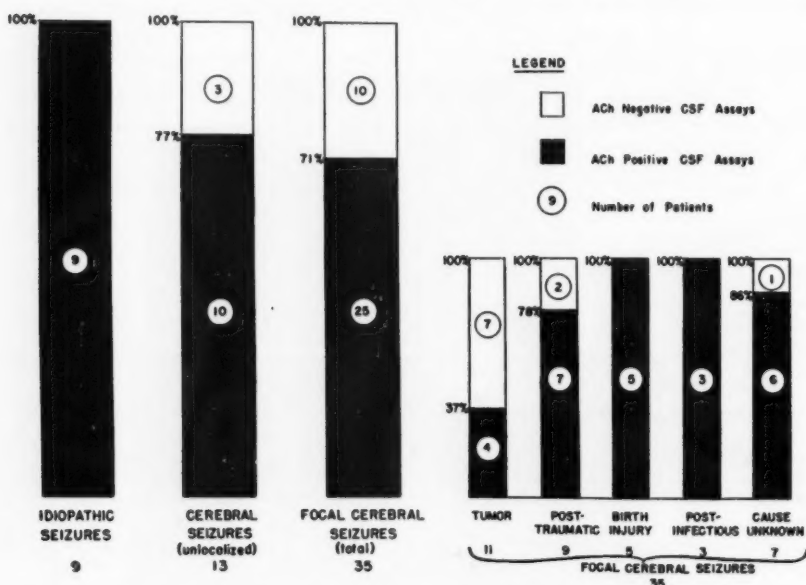CORRELATION OF CSF ACETYLCHOLINE ASSAYS WITH TYPES OF EPILEPSY

FIG. 4.   Correlation of results of cerebrospinal fluid acetylcholine assays with types of epilepsy.

seizure per month.   The relationships of cerebrospinal fluid acetylcholine assays to seizure frequency is illustrated in Fig. 5, A.   In addition the frequency of patients' seizures observed during hospital admission have been correlated with acetylcholine assay results in Fig. 5, B.   From these two graphs it is clear that the majority of cerebrospinal fluid samples positive for acetylcholine occurred in patients with frequent seizures, while the majority of negative assays were in patients with infrequent seizures.

(C) Occurrence of Seizures and Electroencephalographic Abnormalities

In Fig. 6 the correlation of acetylcholine assays with the time of sampling in relation to clinical seizures has been summarized.   The classification of samples was arranged in ictal, postictal, and interictal groups.   Only samples drawn within six hours of an observed clinical seizure were classified as postictal.   All others, except true ictal samples, were designated as interictal. The graph shows that all 13 acetylcholine negative cases were restricted to the interictal group, while all 15 ictal and postictal samples were positive for acetylcholine.   It is of interest to recall that of the six psychiatric cases treated by electric shock convulsant therapy (E.C.T.), which we reported in a previous paper (52), only two were positive for cerebrospinal fluid acetylcholine, although all these samples were taken within 15 min. of the electrically
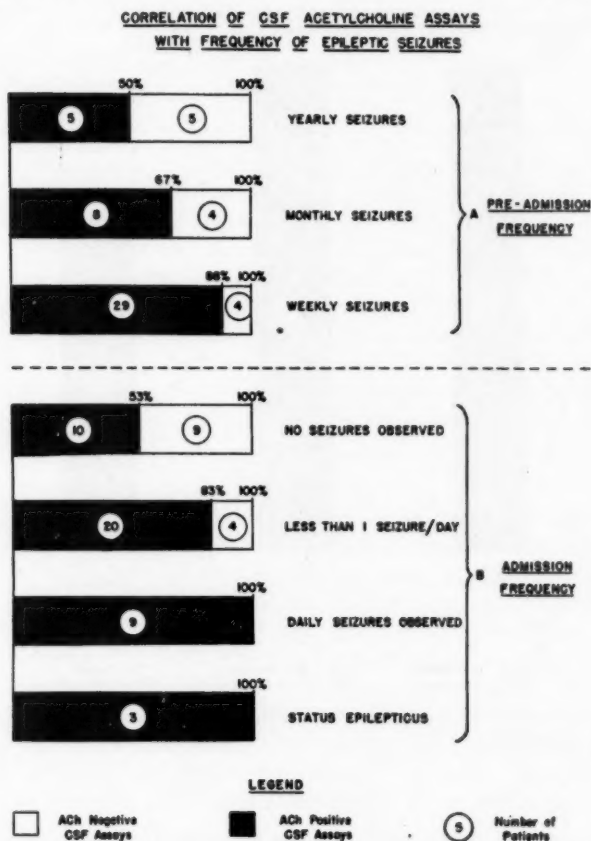
CORRELATION OF CSF ACETYLCHOLINE ASSAYS
WITH FREQUENCY OF EPILEPTIC SEIZURES

FIG. 5.   *Correlation of results of cerebrospinal fluid acetylcholine assays with frequency of clinical epileptic seizures: A—prior to admission (above dotted line) and B—during hospital admission (below dotted line).*

induced generalized seizures.   This difference together with changes seen in the cerebrospinal fluid cholinesterases of the E.C.T. group of patients led us to consider them more typical of cases of craniocerebral trauma than of cases of epilepsy (52).

Because of the importance of subclinical epileptic seizures and the electroencephalographic abnormalities reflecting them, an attempt has been made to correlate the extent of electroencephalographic abnormalities with the results of cerebrospinal fluid acetylcholine assays.   This is summarized in Table III. The classifications given are rather general, based only upon the predominant electroencephalographic abnormalities.   The results shown in Table III are a corollary to those in Fig. 6, since the majority of acetylcholine positive assays

CORRELATION OF CSF ACETYLCHOLINE ASSAYS
WITH OCCURRENCE OF EPILEPTIC SEIZURES
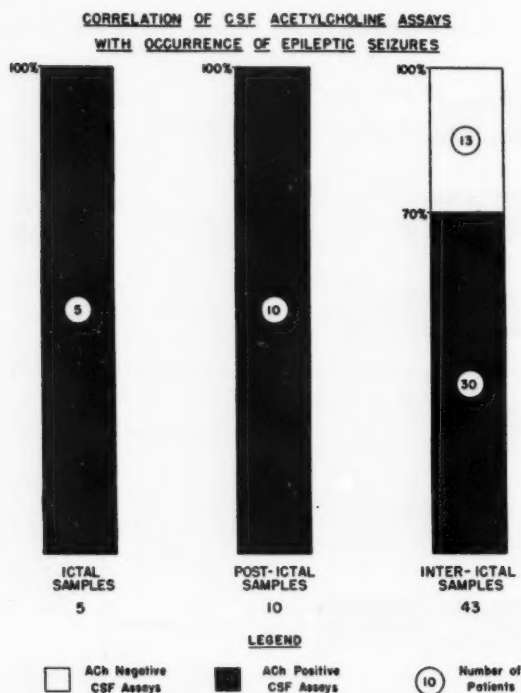
FIG. 6.　*Correlation of results of cerebrospinal fluid acetylcholine assays with occurrence of clinical epileptic seizures.*　(*Samples defined as ictal, during seizure; postictal, within six hours after seizure; interictal, all other samples.*)

TABLE III

CORRELATION OF CEREBROSPINAL FLUID ACETYLCHOLINE ASSAYS WITH PREDOMINANT ELECTRO-
ENCEPHALOGRAPHIC ABNORMALITIES

E.E.G. record abnormality	Total	ACh positive		ACh negative	
1. Paroxysmal bursts or continuous slow and sharp wave activity					
A. Idiopathic three per second wave and spike	8	8		0	
B. Other complexes	17	16		1	
	25	24	(96 %)	1	(4 %)
2. Random or diffuse slow and sharp wave activity	13	8	(62 %)	5	(38 %)
3. Borderline or no abnormality	10	5	(50 %)	5	(50 %)
4. Not tested or record unsatisfactory	9	7		2	
Total	57	44	(77 %)	13	(23 %)

were found to coincide with the more extensive electroencephalographic abnormalities, whereas the negative acetylcholine assays fell into the groups with little or no electroencephalographic abnormalities.

(D) Summary

Acetylcholine assays were made on 119 cerebrospinal fluid samples from 56 epileptic and 53 nonepileptic patients. With the exception of cases of craniocerebral trauma (52), acetylcholine was not found in the nonepileptic patients. Acetylcholine was found in the majority (77 %) of patients with epilepsy at an average level of 1.0 μgm. %, and its presence in the cerebrospinal fluid seemed to relate to the frequency and occurrence of seizures. Cerebrospinal fluid cholinesterase values, medications, assay thresholds, and other factors had no apparent bearing on these findings. It is not clear from this study whether the appearance of acetylcholine in the cerebrospinal fluids of epileptic patients is a cause or result of the seizures or merely a coincidental finding.

Discussion

There must always be an element of doubt as to the identity of a biological substance until it has been chemically isolated. The amounts of acetylcholine appearing in the cerebrospinal fluids of our cases are readily measured by the sensitive biological test employed, but they are too small to make chemical identification feasible. The assay method employed is, however, highly specific for acetylcholine (54). Of the three substances most likely to interfere, *potassium* is without effect in two to three times the concentration found in blood serum (50); *histamine* is without effect in concentrations several hundred times those actually found in cerebrospinal fluid (46); and *adenosine triphosphate* or its derivatives survive the hydrolysis to which test samples have been routinely submitted in order to prove the identity of active substance with acetylcholine (21).

Several reasons for the presence of acetylcholine in the cerebrospinal fluids of epileptic patients could be advanced. Muscular activity during a seizure might in some way be responsible for the appearance of acetylcholine in the cerebrospinal fluid. Blood cholinesterase activity could be expected to deal with such liberated acetylcholine. Furthermore our finding of positive samples from epileptic patients with *petit mal* attacks only and of a correlation between presence of acetylcholine and degree of electroencephalographic abnormality argues against the importance of muscular activity. Since the work of Lorente de N6 (33) it has been known that acetylcholine can be liberated from nervous tissue as a result of trauma. The findings of Bornstein (6) in animals subjected to experimental concussion and our own observations on human cases of craniocerebral trauma (52) confirm this fact. The question arises as to whether the results we have reported here do not represent the recovery of acetylcholine liberated as a result of the trauma to the cerebral cortex of repeated seizures. In our human cases of craniocerebral trauma we found typical changes in cerebrospinal fluid cholinesterases to be more sensitive

indicators of the extent of trauma than the presence of acetylcholine in the cerebrospinal fluid (52).   These cholinesterase changes were entirely lacking in the epileptic patients studied.

We conclude that acetylcholine is present in the cerebrospinal fluids of epileptic patients as a result of some other process than muscular activity or trauma, and that its presence is associated with the occurrence of the epileptic seizures.   The patients in whom we were able to obtain multiple samples proved to be consistently positive.   We are unable to say whether acetylcholine plays a causative role in seizures or whether its appearance in the cerebrospinal fluid is merely a reflection of other events.   It may be argued that, if acetylcholine is concerned with the onset of seizures, atropine should act as a preventive. Physicians formerly used to include belladonna in the therapeutic regime for epilepsy with some good results.   However, the nullifying effect of atropine on acetylcholine when applied to the cortex may be of a low order.   Atropine, for example, has no effect on cholinergic transmission at the myoneural junction of skeletal muscle, whereas it can completely block transmission to visceral muscle.

Recent studies indicate that the potent anticholinesterase, diisopropyl-fluorophosphate (D.F.P.) increases electrical potentials of the cortex and may lead to electroencephalographic seizure patterns (27, 26).   Furthermore the work on specific cholinesterase in the brain suggests that acetylcholine may be intimately concerned with epileptiform cortical discharges (34, 29, 45). In this paper we have demonstrated the presence of acetylcholine in the cerebrospinal fluids of epileptic patients in association with the occurrence of seizures.   A study of epileptogenic brain tissue itself in the light of these observations would now appear to be in order.

Acknowledgment

References

1. ADAM, H. M., McKAIL, R. A., OBRADOR, S., and WILSON, W. C.   J. Physiol. (London), 93 : 45P.   1938.
2. AMMON, R.   Arch. ges. Physiol (Pflügers), 233 : 486.   1933.
3. BABKIN, B. P., SCHACHTER, M., DWORKIN, S., and ROSENBERG, G. M.   Rev. can. biol. 1 : 761.   1942.
4. BERGER, E. N.   Eksptl. Med. Kharkov, 8 (3) : 42.   1941.
5. BEUTNER, R.   In Medical physics.   Edited by O. Glasser.   pp. 35–87.   Year Book Publishers, Inc., Chicago.   1944.
6. BORNSTEIN, M.B.   J. Neurophysiol. 9 : 349.   1946.
7. BORNSTEIN, M. B.   Unpublished data.   1948.
8. BORNSTEIN, M. B. and STERN, K.   Unpublished data.   1948.
9. BRECHT, K.   Klin. Wochschr. 19 : 1087.   1940.
10. BRECHT, K. and KUMMER, H.   Klin. Wochschr. 22 : 741.   1943.
11. BRENNER, C. and MERRITT, H. H.   Arch. Neurol. Psychiat. 48 : 382.   1942.
12. CHANG, H. C., CHIA, K. F., HSU, C. H., and LIM, R. K. S.   Chinese J. Physiol. Rept. Ser. 12 : 1.   1937.

13. CHANG, H. C., HSIEH, W., LI, T., and LIM, R. K. S.   Chinese J. Physiol. Rept. Ser. 13 : 153.   1938.
14. CHATFIELD, P. O. and DEMPSEY, E. W.   Am. J. Physiol. 135 : 633.   1942.
15. CHUTE, A. L., FELDBERG, W., and SMYTH, D. H.   Quart. J. Exptl. Physiol. 30 : 65.   1940.
16. CIPRIANI, A. and BROPHY, D.   J. Lab. Clin. Med. 28 : 1269.   1943.
17. CURE, C. W., RASMUSSEN, T., and JASPER, H.   Arch. Neurol. Psychiat. 59 : 691.   1948.
18. DALE, H. H.   J. Mt. Sinai Hosp. N.Y. 4 : 401.   1938.
19. (a) ELLIOTT, K. A. C.   J. Neurophysiol. 11 : 473.   1948.
    (b) ELLIOTT, K. A. C. and PENFIELD, W.   J. Neurophysiol. 11 : 485.   1948.
20. FELDBERG, W.   Physiol. Revs. 25 : 596.   1945.
21. FELDBERG, W. and HEBB, C.   J. Physiol. (London), 106 : 8.   1947.
22. FELDBERG, W. and SCHRIEVER, H.   J. Physiol. (London), 86 : 277.   1936.
23. FORSTER, F. M.   Arch. Neurol. Psychiat. 54 : 391.   1945.
24. FORSTER, F. M., BOROWSKI, W. J., and McCARTER, R. H.   Federation Proc. 5 : 28.   1946.
25. FORSTER, F. M. and McCARTER, R. H.   Am. J. Physiol. 144 : 168.   1945.
26. FREEDMAN, A. M., BALES, P. D., and HIMWICH, H. E.   Trans. Am. Neurol. Assoc. 73: 64.   1948.
27. GROB, D., HARVEY, A. M., LANGWORTHY, O. R., and LILIENTHAL, J. A.   Bull. Johns Hopkins Hosp. 81 : 257.   1947.
28. HIMWICH, H. E.   J. Am. Med. Assoc. 136 : 870.   1948.
29. HYDE, J. E., BECKETT, S., and GELLHORN, E.   Federation Proc. 6 : 133.   1946.
30. JASPER, H. H.   Ann. Rev. Physiol. 3 : 377.   1941.
31. KAUFMAN, C., MARSHALL, C., and WALKER, A. E.   Arch. Neurol. Psychiat. 58 : 533.   1947.
32. LOEWI, O.   J. Mt. Sinai Hosp. N.Y. 12 : 803; 851.   1945.
33. LORENTE DE Nó, R.   Am. J. Physiol. 121 : 331.   1938.
34. MENDEL, B. and HAWKINS, R. D.   J. Neurophysiol. 6 : 431.   1943.
35. MERRITT, H. H. and BRENNER, C.   Trans. Am. Neurol. Assoc. 67 : 152.   1941.
36. MILLER, F. R.   J. Physiol. (London), 91 : 212.   1937-8.
37. MILLER, F. R., STAVRAKY, G. W., and WOONTON, G. A.   J. Neurophysiol. 3 : 131.   1940.
38. NACHMANSOHN, D.   *In* Vitamins and hormones.   *Edited by* R. S. Harris and K. V. Thimann.   3, p. 337.   Academic Press, Inc., New York.   1945.
39. NACHMANSOHN, D.   Ann. N.Y. Acad. Sci. 47 : 395.   1946.
40. NACHMANSOHN, D. and ROTHENBERG, M. A.   J. Biol. Chem. 158 : 653.   1945.
41. NOCHIMOWSKI, C.   J. physiol. J. path. gén. 35 : 746.   1937.
42. ODOM, G., RUSSEL, C. K., and McEACHERN, D.   Brain, 66 : 1.   1943.
43. PARROT, J. L. and LEFEBVRE, J.   Compt. rend. soc. biol. 137 : 662.   1943.
44. PENFIELD, W.   Arch. Neurol. Psychiat. 60 : 107.   1948.
45. POPE, A., MORRIS, A. A., JASPER, H., ELLIOTT, K. A. C., and PENFIELD, W.   Research Pubs. Assoc. Research Nervous Mental Disease, 26 : 218.   1947.
46. ROSE, B.   Personal communication.   1948.
47. SJÖSTRAND, T.   J. Physiol. (London), 90 : 41P.   1937.
48. TOWER, D. B.   Can. J. Research, E, 27 : 20.   1949.
49. TOWER, D. B.   Unpublished data. 1948.
50. TOWER, D. B. and McEACHERN, D.   Can. J. Research, E, 26 : 183.   1948.
51. TOWER, D. B. and McEACHERN, D.   Can. J. Research, E, 27 : 132.   1949.
52. TOWER, D. B. and McEACHERN, D.   Can. J. Research, E, 27 : 105.   1949.
53. WAIT, R. B.   Biol. Bull. 85 : 79.   1943.
54. WELSH, J. H.   Pharmacology of isolated ventricle of *Venus mercenaria*.   In preparation.
55. WILLIAMS, D. and RUSSELL, W. R.   Lancet, 1 : 476.   1941.

THE CONTENT AND CHARACTERIZATION OF CHOLINESTERASES IN HUMAN CEREBROSPINAL FLUIDS[1]

By Donald B. Tower and Donald McEachern

Abstract

Determinations of cerebrospinal fluid cholinesterase activities and fractions were made on 114 samples from 104 neurological and neurosurgical patients. Details of methods are given. Average rate of acetylcholine hydrolysis was 0.39 cu. mm. carbon dioxide per ml. CSF per min. or approximately 0.5% of the cholinesterase activity of an equal volume of blood or serum. Characterization of cholinesterase fractions, using mecholyl and benzoylcholine as substrates, showed human cerebrospinal fluid to contain specific cholinesterase with a small component of unspecified cholinesterase. Differences of blood and subdural fluid fraction patterns are demonstrated.

No correlation of cholinesterase values with cerebrospinal fluid cell counts were found. Attempts to correlate cholinesterase values with cerebrospinal fluid Pandy tests and protein levels were inconclusive, although samples with positive Pandy tests and abnormally high protein levels showed a tendency toward decrease in specific and increase in unspecified cholinesterases. No correlation of cerebrospinal fluid cholinesterase values with clinical diagnoses was demonstrated with the exception of cases of craniocerebral trauma and cases treated by electric shock convulsant therapy, where a decrease in specific and a marked increase in unspecified cholinesterase fractions were observed. At acetylcholine substrate concentrations in the "physiological range" (0.01 to 10.0 μgm. %) cerebrospinal fluid cholinesterase activities were markedly reduced or absent.

Introduction

The present study was undertaken in an attempt to provide a clearer understanding of the role of cerebrospinal fluid cholinesterases in normal and disease processes.

Several reports on cerebrospinal fluid cholinesterases have appeared in the literature. In 1930 Plattner and Hinter (37) found cerebrospinal fluid cholinesterase activity to be 1/20 to 1/25 that of blood serum cholinesterase. Vahlquist (49) and Stedman and Stedman (42) were unable to demonstrate any cholinesterase activity in spinal fluid. Ginsberg *et al.* (21) also questioned its presence there. However, Altenburger (3) reported spinal fluid cholinesterase activity to be 1/250 that of the blood. Bender (5) found 1 to 2% of the activity present in blood serum. He noted that the spinal fluid cholinesterase activity was so low that apparently not all the acetylcholine that might be liberated into it would be destroyed. Pinotti and Tanfani (36), Birkhäuser (7), and Glasson and Mutrux (22) have also found cholinesterases present in cerebrospinal fluid with about 1/200 the activity of blood and blood serum. A characterization of the types of cholinesterases present was made by Glasson and Mutrux (22). They concluded that cerebrospinal fluid contained specific cholinesterase in a concentration about 1/200 that of red cells, together with a smaller quantity of "ali-esterase." A preliminary

report by Reiss and Hemphill (39) confirmed these findings by a somewhat different method.   The four most recent studies cited have shown that there was considerable variation in the cholinesterase activities of individual spinal fluid samples, which did not seem to be well correlated with cellular reaction or protein content of the fluids.   No reports have been made on large groups of patients.

Of the several methods for determining cholinesterases the one most commonly employed is the Warburg method of Ammon (4a), in which substrate esters are split by the enzyme to liberate the substrate acid.   In the presence of bicarbonate the acid liberates carbon dioxide gas, which can be measured manometrically.   Modifications of this principle for very small quantities have been used to measure the liberated acid directly by a micro-titrimetric method (23), to measure the gas evolution by the "Cartesian diver" technique (8), and to measure the residual acetylcholine after completion of the reaction (14).   We have used a modification of the method of Ammon (4a) reported by Odom *et al.* (34).

Characterization of cholinesterases depends on the demonstration of two general types of cholinesterases in the blood by Alles and Hawes (2), which was subsequently confirmed by Richter and Croft (40), Mendel and Rudney (31) and Mendel, Mundell, and Rudney (30).   By the use of mecholyl (acetyl-β-methylcholine), benzoylcholine, and other choline and noncholine esters, certain differences in patterns of response were found, which were characteristic of the different types of cholinesterases.   The theoretical basis of this method is schematically summarized in Fig. 1.   It depends on the following

CHARACTERIZATION of CHOLINESTERASES in HUMAN CEREBROSPINAL FLUID

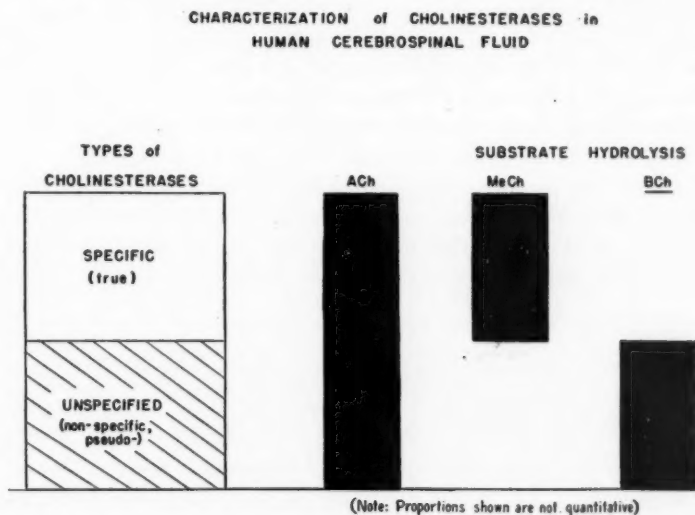(Note: Proportions shown are not quantitative)

Fig. 1.   *Theoretical basis of qualitative characterization of cholinesterases.   (ACh = acetylcholine; MeCh = mecholyl; BCh = benzoylcholine.)*

observations: (*a*) acetylcholine (ACh) is hydrolyzed by both esterase fractions; (*b*) mecholyl (MeCh) is selectively hydrolyzed by specific (or true) cholinesterase fractions; and (*c*) benzoylcholine (BCh) is selectively hydrolyzed by the unspecified (nonspecific or pseudo-) cholinesterase fractions (30, 32).* Since these are qualitative distinctions, Nachmansohn and Rothenberg (32) devised a method for making quantitative comparisons. The rates of hydrolysis of various substrates were reported as ratios of the rate of acetylcholine hydrolysis by the same sample (with ACh/ACh always equal to 100). By this method the two types of cholinesterases could be defined by their MeCh and BCh ratios as follows: *specific cholinesterase:* MeCh/ACh = 30-60; BCh/ACh = ± 1 and. *unspecified cholinesterase:* MeCh/ACh = 1-5; BCh/ACh = 50-250 (32). A modification of this method has been used for characterization of cerebrospinal fluid cholinesterases in this study.

Materials and Methods

Cerebrospinal fluid samples (114 samples) from 104 neurological and neurosurgical patients were studied. Three samples proved to be subdural fluids and are considered separately from the 111 CSF samples. Of the CSF samples, 80% were obtained during pneumoencephalography, the remainder during ventriculography (4%) or lumbar puncture (16%). Care was taken to mix the total fluid volume drained from the subarachnoid spaces and ventricles before taking samples for study.

All CSF samples were examined for cell count, Pandy reaction, protein level, and acetylcholine as well as for cholinesterases. Protein determinations were done by the microturbidimetric method of Cipriani and Brophy (16). Acetylcholine assays were done by our modification of the method of Wait (50, 46) and will be reported elsewhere (47, 48, 18). The initial 30 samples were also analyzed for sugar, chlorides, and Lange curve reaction, but since it became evident that these determinations had no bearing on the main study they were discontinued.

CSF samples for cholinesterase determinations were simply refrigerated at 5° to 10° C. until used. Under such conditions the enzyme has been shown to be stable and to remain suitable for testing for several weeks (25). Our method for determining cholinesterase activity and fractions is as follows.

(1) *Apparatus*

A conventional Warburg apparatus with an electrically driven bath stirrer and shaker panel (set at 116 to 120 oscillations per min.) and a thermostatically controlled constant temperature bath (set at 37.5° C.) was used.

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* *The terminology of cholinesterase fractions has become rather confusing. It has seemed to us that the most reasonable terminology is that used throughout this paper, namely, specific cholinesterase for that fraction specific to the nervous system and neuromuscular tissues and unspecified cholinesterase for that fraction found in other body tissues and fluids for which no specific function has been discovered. The terms "true," "nonspecific" or "pseudo-" suggest connotations for which no evidence has yet been offered.*

Calibrated manometers filled with Brodie's solution and fitted with calibrated conical Warburg flasks completed the apparatus.

(2) *Materials*

CSF volume of 1.5 ml. per flask was chosen on the basis of the desired rate of gas evolution for enzyme activity 0.5% that of blood serum (34). Substrate concentrations, experimentally derived on the basis of the reports of Nachmansohn and Rothenberg (32) are given in Table I. Bicarbonate solution, experimentally modified from Nachmansohn and Rothenberg (32) and Odom *et al.* (34), is also given in Table I.

TABLE I

A.   *Substrate concentrations used**

Substrate	Initial concentration in %	Final molar concentration
Acetylcholine	0.5	6.9×10^{-3}
Mecholyl	1.0	1.3×10^{-2}
Benzoylcholine	1.0	1.0×10^{-2}

B.   *Bicarbonate solution used**

Salt	Initial concentration in %	Parts	Final molar concentration
NaCl	0.9	80	3.1×10^{-2}
NaHCO₃	1.3	16	6.2×10^{-3}
MgCl₂ . 6H₂O	2.24	4	1.1×10^{-3}

* For use with 0.5 ml. substrate–bicarbonate solution and 1.5 ml. enzyme solution = fluid reaction volume of 2.0 ml.

(3) *Procedure*

CSF (1.5 ml.) was pipetted into the body of each flask and 0.5 ml. of substrate in bicarbonate solution (Table I) was placed in the side bulb. Duplicate runs were made on all samples reported here. A third flask was included in each sample run as a combined thermobarometer and auto-hydrolysis indicator. All flasks were saturated with 95% nitrogen – 5% carbon dioxide gas, placed in the constant temperature bath, and shaken for 20 min. to achieve equilibrium. Zero readings were taken, the substrate and enzyme solutions thoroughly mixed, and the reaction followed. Manometer readings were taken at five minute intervals for the first 20 min. and at 10 to 20 min. intervals thereafter for 60 min. reaction time. (The first 50 runs were followed for 80 min. and showed no dropping off of the plotted

rate of hydrolysis.) After applying corrections for the thermobarometer–autohydrolysis deviations and the flask constants, the resulting standard gas volumes were plotted graphically against time and the rates of substrate hydrolysis derived from the slopes of the lines plotted. Cholinesterase fraction patterns were calculated from the hydrolysis rates so determined (Figs. 2, 3, 4).

1. *Human Cerebrospinal Fluid Cholinesterase Content (Activity) and Fraction Patterns*

The cholinesterase values obtained from examination of 111 cerebrospinal fluid samples from 102 patients are given in Table II. Averages of the results

TABLE II

RANGE OF CEREBROSPINAL FLUID CHOLINESTERASE VALUES

Substrate	Rates of hydrolysis (in cu. mm. CO_2/ml./min.)	Substrate ratios (S/ACh)
Acetylcholine	0.12 – 0.89	100
Mecholyl	0.02 – 0.30	8 – 52
Benzoylcholine	0.00 – 0.19	0 – 43

on these patients are illustrated in Fig. 2. It may be seen that the average rate of acetylcholine (ACh) hydrolysis was 0.39 cu. mm. carbon dioxide per
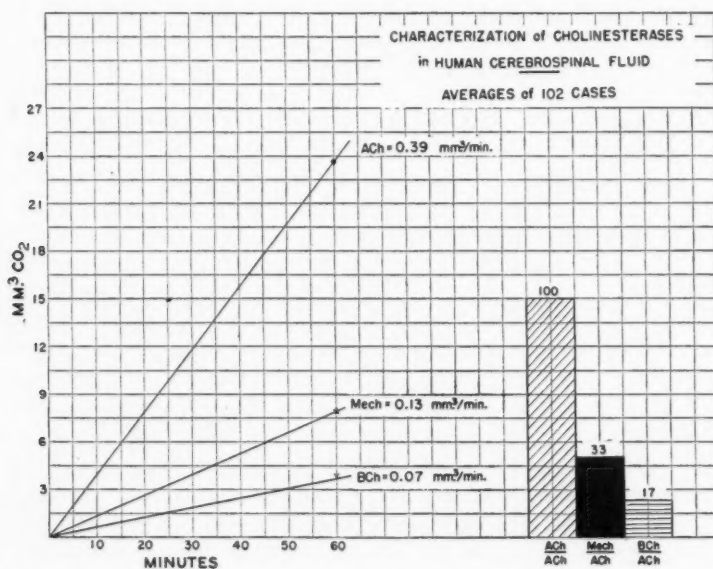

FIG. 2. *Cerebrospinal fluid cholinesterase values (For legend see Fig. 3. Rates are given per ml. of CSF).*

ml. CSF per min., and that the average substrate ratios were 33 for MeCh/ACh and 17 for BCh/ACh.   By the definition of Nachmansohn and Rothenberg (32) these values characterize the cerebrospinal fluid cholinesterases as consisting mostly of a specific cholinesterase fraction with a small unspecified cholinesterase fraction.   Our findings agree with the reports of Birkhäuser (7) (average ACh activity of 0.34 cu. mm. carbon dioxide per ml. CSF per min. in 46 cases) and of Glasson and Mutrux (22) (average ACh activity of 0.42 cu. mm. carbon dioxide per ml. CSF per min. in 60 cases).   Reiss and Hemphill (39), using a method similar to ours in 42 mental patients, reported a comparable range of values, although their average rates of hydrolysis were somewhat higher (for ACh 0.71 to 0.79 cu. mm. carbon dioxide per ml. CSF per min.).   Because of somewhat different conditions in their experiments it is not possible to compare results further.

Cholinesterase levels in normal blood samples studied by us agreed well with those reported by Odom *et al.* (34) and Nachmansohn and Rothenberg (32).   One such sample is illustrated in Fig. 3.   The distinction of the two
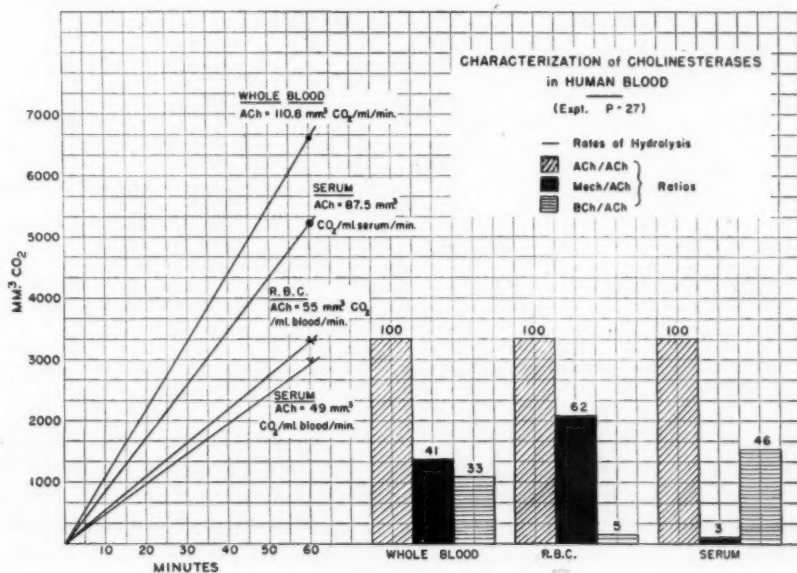
FIG. 3.   *Blood cholinesterase values.*

principal types of cholinesterases known to exist in human blood was shown by the specific pattern of the red blood cells and the unspecified pattern of the serum.   The mixture of these two fractions produced a pattern for whole blood that was analogous to that seen for cerebrospinal fluid (Fig. 2).   The comparison of the rates of hydrolysis for samples of blood with the average rates for cerebrospinal fluids showed that cerebrospinal fluid cholinesterases

had an average activity of about 1/225 (0.45%) that of an equal volume of serum, and 1/285 (0.35%) that of an equal volume of whole blood. No attempt was made to correlate blood and cerebrospinal fluid cholinesterase values in the same patient.

In Fig. 4 average cholinesterase values from three samples of subdural fluids obtained from subdural effusions (during ventricular puncture) contrast with the cerebrospinal fluid values obtained in other cases. The subdural
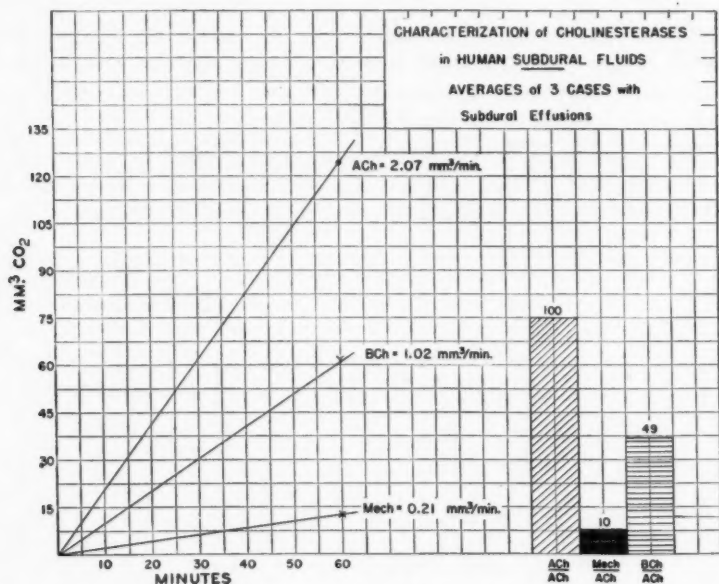
Fig. 4. *Subdural fluid cholinesterase values (For legend see Fig. 3. Rates are given per ml. of fluid).*

fluids resembled blood serum in their cholinesterase fraction patterns, although the activity was about 2% that of the serum. Cholinesterase activity of subdural fluids (with ACh as substrate) was five times the average cerebrospinal fluid activity. These differences of subdural fluids have been of some practical value in detecting probable contamination of cerebrospinal fluid samples with subdural fluid. In our study of cases of craniocerebral trauma this has assumed considerable importance (47).

2. Correlation of Cholinesterase Values with Other Constituents of Human Cerebrospinal Fluids

Cell counts were done on 100 of the 111 cerebrospinal fluid samples. In Table III comparisons of the effects of normal and increased numbers of white cells and red cells in the cerebrospinal fluid with cholinesterase values

TABLE III

COMPARISON OF CEREBROSPINAL FLUID CELL COUNTS
WITH CHOLINESTERASE VALUES

A. *White blood cells*

	0-6 WBC	Over 6 WBC
Number of samples	84	16
Average WBC/ml.	—	20
ChE rates (cu. mm. CO_2/ml./min.)		
ACh	0.39	0.39
MeCh	0.13	0.12
BCh	0.07	0.07
ChE ratios		
MeCh/ACh	33	31
BCh/ACh	17	17

B. *Red blood cells*

	0-50 RBC	50-100 RBC	100-500 RBC	Over 500 RBC
Number of samples	78	9	11	2
Average RBC/ml.	9-10	73	134	640
ChE rates (cu. mm. CO_2/ml./min.)				
ACh	0.39	0.46	0.34	0.34
MeCh	0.13	0.13	0.12	0.12
BCh	0.07	0.08	0.07	0.06
ChE ratios				
MeCh/ACh	33	28	35	35
BCh/ACh	17	17	21	18

have been summarized.   No significant differences were apparent.   Ginsberg
et al. (21) were unable to find any cholinesterase activity in white blood cells.
Calculation of the volume of carbon dioxide liberated by each red cell showed
that a contamination of 2000 red cells per ml. would be necessary to produce a
significant increase in cholinesterase activity (more than 1.0 cu. mm. carbon
dioxide per hour).*   None of the cerebrospinal fluid samples in our study
showed a pronounced pleocytosis, and in no case did the red cell count exceed
710 per ml.   Possibly, in cases of acute meningitis, differences would be
found (7), but these could be due to the effects of altered permeability of the
blood–brain barrier as a result of inflammation rather than to pleocytosis

* *The rate of ACh hydrolysis by red cells (Cf. Fig. 3) was 55 cu. mm. carbon dioxide per ml.
RBC per minute with a red cell count of 5.5 million per ml.   This is equivalent to approximately
0.0006 cu. mm. carbon dioxide per red cell per hour.*

per se. In a similar fashion the slight decrease in the specific cholinesterase fraction seen with red cell counts of 50 and over (Table III) may have been due to serum added with the cells. Therefore, we have concluded that in the samples studied the presence of cells in the cerebrospinal fluid has not been a factor influencing cholinesterase values.

Pandy tests and *protein determinations* were carried out on 104 and 108 of the total of 111 cerebrospinal fluid samples, respectively. Comparisons with cholinesterase values are given in Table IV. There is a suggestion that with

TABLE IV A

COMPARISON OF CEREBROSPINAL FLUID PANDY REACTIONS
WITH CHOLINESTERASE VALUES*

	0 Reaction	± Reaction	1+ Reaction	2+ Reaction
Number of samples	57	18	19	9
ChE rates (cu. mm. CO_2/ml./min.)				
ACh	0.38	0.43	0.38	0.41
MeCh	0.13	0.15	0.13	0.11
BCh	0.06	0.07	0.07	0.09
ChE ratios				
MeCh/ACh	34	34	34	27
BCh/ACh	16	16	19	22

* The one sample with 3+ reaction is omitted.

TABLE IV B

COMPARISON OF CEREBROSPINAL FLUID PROTEIN LEVELS
WITH CHOLINESTERASE VALUES

	0-20 mgm. %	20-35 mgm. %	35-50 mgm. %	Over 50 mgm. %
Number of samples	26	45	20	17
Average protein level (mgm. %)	15.8	25.7	42.6	76.9
ChE rates (cu. mm. CO_2/ml./min.)				
ACh	0.32	0.39	0.43	0.43
MeCh	0.11	0.13	0.14	0.12
BCh	0.04	0.07	0.07	0.10
ChE ratios				
MeCh/ACh	34	33	33	28
BCh/ACh	13	17	16	23

more strongly positive Pandy reactions there is a relative decrease in the specific cholinesterase fraction (MeCh activity) with a corresponding increase in the unspecified fraction (BCh activity). At abnormal protein levels (over 50 mgm. %) there was also a relative increase in the unspecified cholinesterase fraction and a decrease in the specific fraction. The Pandy test does not necessarily correlate with the cerebrospinal fluid protein level, but it is usually

positive when protein values are increased.   Cohn *et al.* (17) have reported that serum (unspecified) cholinesterase is carried in Cohn's plasma protein fraction IV-6, which is 95% alpha$_2$ globulin and 5% albumin.   Our results suggest that a significant portion of the increase in protein may be globulins, admitted because of altered permeability of the meningeal and cerebral vessels.   The difficulty in attempting to correlate protein and cholinesterase values in the cerebrospinal fluids lies in the facts that both constituents represent mixtures of at least two fractions and that there are no satisfactory methods for studying the individual fractions separately.   Because of the small number of abnormal samples it has not seemed justifiable to emphasize the possible significance of correlations of cholinesterase values with Pandy tests and protein levels further.

3. *Correlation of Cerebrospinal Fluid Cholinesterase Values with Clinical Diagnoses*

Average cerebrospinal fluid cholinesterase values for the various diagnostic groups are given in a previous paper (47, Fig. 1).   Our primary interest has been investigating possible biochemical abnormalities in epilepsy, so that 47% of the patients fell into that group.   Because of the small numbers in each of the other diagnostic groups no separate tabulations seemed justified with the exception of those shown.   Rates of acetylcholine hydrolysis and cholin-esterase fraction patterns for the various diagnostic groups were not signi-ficantly different with two exceptions.   In cases of craniocerebral trauma the rate of acetylcholine hydrolysis was less than in other groups.   And in these cases, as well as those treated by electric shock convulsant therapy (E.C.T.), there was a decrease in the specific cholinesterase fraction (MeCh activity) and a marked increase in the unspecified cholinesterase fraction (BCh activity). Of the samples, 70% in these two groups showed reversals of the usual cholin-esterase fraction patterns, whereas less than 10% of the remainder showed any tendency toward such reversals.   There was a definite correlation between the extent of the cholinesterase abnormalities and the degree of trauma. From these observations, together with electroencephalographic, clinical and cerebrospinal fluid acetylcholine findings, we have considered it justifiable to differentiate cases of craniocerebral trauma from the other patients studied. A detailed report of these cases will appear elsewhere (47).   Detailed analysis failed to show any significant correlation of cerebrospinal fluid cholinesterase values with epilepsy or the epileptic seizure in our cases.*

4. *Cerebrospinal Fluid Cholinesterase Activity at Substrate Concentrations in the "Physiological Range"*

The finding of 0.02 to 100+ μgm. % of acetylcholine in the cerebrospinal fluids of epileptic and craniocerebral trauma patients (47, 48, 18) in the

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* *Because Pope et al. (38) had reported increased cholinesterase activity in tissues from experimentally produced focal epileptogenic lesions, it seemed that some changes might be expected in cerebrospinal fluid cholinesterases as well.   No differences were uncovered among the types of epilepsy, seizure frequency, types of spinal fluid samples, presence or absence of acetylcholine in samples, or the level of acetylcholine present in the cerebrospinal fluid (18, 48).*

presence of cholinesterase activities capable of hydrolyzing an average of 3 μgm. of acetylcholine per ml. per min. (300 μgm. % per min.)* seemed paradoxical. Bender (5) had predicted that probably not all the acetylcholine present in the cerebrospinal fluid would be inactivated by the concentrations of cholinesterases present. Nachmansohn and Rothenberg (32) have shown that the specific cholinesterase of nervous tissue has an optimal substrate concentration (about 10^{-2} molar for ACh) and that at higher or lower substrate concentrations the cholinesterase activity drops off markedly. In our studies the final acetylcholine concentrations used in cholinesterase determinations were about 125,000 times the average of 1.0 μgm. % acetylcholine in epileptic cerebrospinal fluid samples (48, 18).

Accordingly a series of experiments was carried out to test the cholinesterase activities of cerebrospinal fluids at optimal substrate concentrations and at concentrations down to those actually encountered in clinical experience. Fig. 5 shows the curve derived from these experiments. At substrate con-
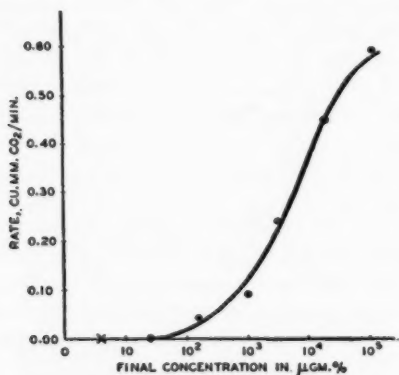

FIG. 5. *Curve of variation in cerebrospinal fluid cholinesterase activity with substrate concentrations. Rates of ACh hydrolysis plotted against final substrate concentrations. (• = Warburg method; × = Venus heart method.)*

centrations below 150 μgm. % the manometric method is no longer able to detect cholinesterase activity. For this range a modification of the method of Bullock *et al.* (14) was used.** From this set of experiments it seems clear

* *Cholinesterase activity of 1.0 cu. mm. carbon dioxide per ml. CSF per min. is equivalent to hydrolysis of 7.3 μgm. of acetylcholine per ml. CSF per min.*

** *Amounts of acetylcholine to give final concentrations of 0.2 to 4.0 μgm. % were added to cerebrospinal fluids, previously shown to be free of acetylcholine. The samples were incubated at 37.5° C. for one hour. Control samples were taken at the start of the experiments and at 30 and 60 min. intervals of incubation time. Residual acetylcholine concentrations were preserved by the method of Tower (45) until they could be assayed on the isolated ventricle of Venus mercenaria (46). No attempt was made to control all the factors involved such as pH, loss with bicarbonate and the like, but results in 12 experiments were sufficiently consistent to make us feel that the method served the purposes of this study.*

that at substrate concentrations in what may be termed the "physiological range" the cholinesterase activity of cerebrospinal fluids in most cases was zero. These findings are consistent with those of Nachmansohn and Rothenberg (32, Fig. 2). We conclude that the activities of cerebrospinal fluid cholinesterases were not significant factors in assays for cerebrospinal fluid acetylcholine, which we report elsewhere (47, 48, 18).*

Discussion

That specific cholinesterase is the principal fraction of human cerebrospinal fluid is of interest. Mendel and Rudney (31) and Nachmansohn and Rothenberg (32) have shown that the cholinesterase of nervous tissue is specific cholinesterase. It is noteworthy that the enzyme in cerebrospinal fluid, which bathes this tissue, should also be specific cholinesterase. This may throw further light on the sources of cerebrospinal fluid and its usefulness in reflecting events in the central nervous system. Since the red cell is the only extraneural source of specific cholinesterase (31, 40, 32), it seems probable that the source of specific cholinesterase in cerebrospinal fluid is the central nervous system. The enzyme is a protein, and it follows that one fraction at least of the spinal fluid protein is contributed by nervous tissue. This is not a new concept, and certainly the studies of Spiegel-Adolph (43), Spiegel *et al.* (44), Sawyer (41), Hyden (27, 28), and Hamberger and Hyden (24) support this view.

Since there is little or no unspecified cholinesterase in nervous tissue, its presence in the cerebrospinal fluid suggests an extraneural source and the same for the protein fraction in which it is carried. The finding of increased unspecified cholinesterase fractions with positive Pandy tests and abnormally high protein levels (particularly in cases of craniocerebral trauma) complement one another to indicate a probable increase in the contribution of the extraneural protein fraction or fractions, which is presumably due to alterations in permeability of the blood–brain barrier. The recent report of Kabat *et al.* (29) fits in with this concept. In addition in craniocerebral trauma the specific fraction of cerebrospinal fluid cholinesterases is reduced. These observations have led us to suggest that cerebrospinal fluid cholinesterases may be sensitive indicators of certain abnormalities of central nervous system function (47).

One other interesting problem is revived by this study, namely, the problem of cholinesterase excess in the central nervous system (20). The presence of amounts of specific cholinesterase apparently more than sufficient to hydrolyze all the acetylcholine that might be liberated into it has puzzled neurophysiologists. Free acetylcholine has been reported in the cerebrospinal fluid by a number of investigators (19, 1, 33, 15, 12, 6, 13, 35, 9, 10, 11, 26, 47, 48, 18). The explanation of this apparent paradox, as far as the spinal fluid cholinesterases are concerned, has been offered in this study. It depends on the

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* *Since completion of this paper the exhaustive review of cholinesterases by Augustinsson (4(b)) has been published. The experimental observations reported by him for tissue and blood cholinesterases corroborate and expand the findings and conclusions presented here for cerebrospinal fluids.*

fact that at suboptimal concentrations of substrate in the "physiological range" specific cholinesterase activity is markedly reduced or absent. Nachmansohn and Rothenberg (32) have shown that a similar situation exists in nervous tissue, but this seems to have been overlooked. The results of our experiments suggest the importance of the same approach to the study of specific cholinesterase activity of nervous tissue.

We raise the possibility that the apparent excess of cholinesterase is not an excess at all, but that it is the amount necessary to deal with suboptimal acetylcholine concentrations usually encountered. The fact that the optimal substrate concentration for the enzyme is far above conceivable physiological ranges is intriguing. Could it be that the enzymes serve another purpose, that of dealing with massive amounts of acetylcholine liberated under certain pathological conditions? It might be logical to think of specific cholinesterase as possessing these dual functions to cover an extensive range of substrate concentrations (47). Certainly a critical review of the role of specific cholinesterase in neuronal activity is indicated.

Acknowledgment

References

1. ADAM, H. M., McKAIL, R. A., OBRADOR, S., and WILSON, W. C.   J. Physiol. (London), 93 : 45P.   1938.
2. ALLES, G. A. and HAWES, R. C.   J. Biol. Chem. 133 : 375.   1940.
3. ALTENBURGER, H.   Klin. Wochschr. 16 : 398.   1937.
4 (a). AMMON, R.   Arch. ges. Physiol. (Pflügers), 233 : 486.   1933.
  (b). AUGUSTINSSON, K-B.   Acta Physiol. Scand. 15 (Suppl. 52) : 1.   1948.
5. BENDER, M. B.   Am. J. Physiol. 126 : 180.   1939.
6. BERGER, E. N.   Eksptl. med. Kharkov, 8 (3) : 42.   1941.
7. BIRKHÄUSER, H.   Schweiz. Arch. Neurol. Psychiat. 46 : 185.   1941.
8. BOELL, E. J.   J. Cellular Comp. Physiol. 25 : 75.   1945.
9. BORNSTEIN, M. B.   J. Neurophysiol. 9 : 349.   1946.
10. BORNSTEIN, M. B.   Unpublished data.   1948.
11. BORNSTEIN, M. B. and STERN, K.   Unpublished data.   1948.
12. BRECHT, K.   Klin. Wochschr. 19 : 1087.   1940.
13. BRECHT, K. and KUMMER, H.   Klin. Wochschr. 22 : 741.   1943.
14. BULLOCK, T. H., GRUNDFEST, H., NACHMANSOHN, D., and ROTHENBERG, M. A.   J. Neurophysiol. 10 : 63.   1947.
15. CHUTE, A. L., FELDBERG, W., and SMYTH, D. H.   Quart. J. Exptl. Physiol. 30 : 65.   1940.
16. CIPRIANI, A. and BROPHY, D.   J. Lab. Clin. Med. 28 : 1269.   1943.
17. COHN, E. J., STRONG, L. E., HUGHES, W. L., MULFORD, D. L., ASHWORTH, J. M., MELIN, M., and TAYLOR, H. L.   J. Am. Chem. Soc. 68 : 459.   1946.
18. CONE, W. V., TOWER, D. B., and McEACHERN, D.   Trans. Am. Neurol. Assoc. 73 : 59. 1948.
19. FELDBERG, W. and SCHRIEVER, H.   J. Physiol. (London), 86 : 277.   1936.
20. GERARD, R. W.   Ann. N.Y. Acad. Sci. 47 : 575.   1946.
21. GINSBERG, R., KOHN, R., and NECHELES, H.   Am. J. Digestive Diseases Nutrition, 4 : 154.   1937.
22. GLASSON, B. and MUTRUX, S.   Helv. Physiol. et Pharmacol. Acta, 4 : C12.   1946.
23. GLICK, D.   J. Gen. Physiol. 21 : 289.   1938.

24. HAMBERGER, C-A. and HYDEN, H.   Acta Oto-Laryngol. Suppl. No. 61.   1945.
25. HAWKINS, R.   Removal of acetylcholine by cholinesterase injection and effect thereof on nerve impulse transmission.   Univ. Toronto, PhD. Thesis.   1943.
26. HIMWICH, H. E.   J. Am. Med. Assoc. 136 : 870.   1948.
27. HYDEN, H.   Acta Physiol. Scand. 6 (Suppl. 17) : 1.   1943.
28. HYDEN, H.   Symposium Soc. Exptl. Biol. 1 : 152.   1947.
29. KABAT, E. A., GLUSSMAN, M., and KNAUB, V.   Am. J. Med. 4 : 653.   1948.
30. MENDEL, B., MUNDELL, D. B., and RUDNEY, H.   Biochem. J. 37 : 473.   1943.
31. MENDEL, B. and RUDNEY, H.   Biochem. J. 37 : 59.   1943.
32. NACHMANSOHN, D. and ROTHENBERG, M. A.   J. Biol. Chem. 158 : 653.   1945.
33. NOCHIMOWSKI, C.   J. physiol. path. gén. 35 : 746.   1937.
34. ODOM, G., RUSSEL, C. K., and McEACHERN, D.   Brain, 66 : 1.   1943.
35. PARROT, J. L. and LEFEBVRE, J.   Compt. rend. soc. biol. 137 : 662.   1943.
36. PINOTTI, O. and TANFANI, L.   Riv. patol. nervosa e mentale, 53 : 181.   1939.
37. PLATTNER, F. and HINTER, H.   Arch. ges. Physiol. (Pflügers), 255 : 19.   1930.
38. POPE, A., MORRIS, A. A., JASPER, H., ELLIOTT, K. A. C., and PENFIELD, W.   Research Pubs. Assoc. Research Nervous Mental Disease, 26 : 218.   1947.
39. REISS, M. and HEMPHILL, R. E.   Nature, 161 : 18.   1948.
40. RICHTER, D. and CROFT, P. G.   Biochem. J. 36 : 746.   1942.
41. SAWYER, C. H.   Am. J. Physiol. 146 : 246.   1946.
42. STEDMAN, E. and STEDMAN, E.   Biochem. J. 29 : 2107.   1935.
43. SPIEGEL-ADOLF, M.   J. Nervous Mental Disease, 89 : 311.   1939.
44. SPIEGEL, E. A., SPIEGEL-ADOLF, M., WYCIS, H. T., and MARKS, M.   Research Pubs. Assoc. Research Nervous Mental Disease, 26 : 84.   1947.
45. TOWER, D. B.   Can. J. Research, E, 27 : 20.   1949.
46. TOWER, D. B. and McEACHERN, D.   Can. J. Research, E, 26 : 183.   1948.
47. TOWER, D. B. and McEACHERN, D.   Can. J. Research, E, 27 : 105.   1949.
48. TOWER, D. B. and McEACHERN, D.   Can. J. Research, E, 27 : 120.   1949.
49. VAHLQUIST, B.   Skand. arch. physiol. 72 : 133.   1935.
50. WAIT, R. B.   Biol. Bull. 85 : 79.   1943.

SOME EFFECTS OF DIISOPROPYL FLUOROPHOSPHATE (DFP) AND FLUOROACETATE ON THE CENTRAL NERVOUS SYSTEM[1]

By Andrew Kelen and Donald McEachern

Abstract

Attempts were made to produce convulsions in cats by intracarotid and intracisternal injections of diisopropyl fluorophosphate (DFP). No convulsions were obtained in either instance. Systemic symptoms appeared, however, and plasma cholinesterase was sharply reduced. Intracarotid DFP produced prolonged ipsilateral myosis and salivation. E.E.G. changes on the side of the injection were found in one of two trials. Intracisternal DFP caused prolongation (threefold) of the pentothal anesthesia. The animals, upon awakening, showed a temporary loss of sensation in the forelimbs and cornea with weakness of the hind limbs and a waddling gait. Intracisternal fluoroacetate produced curious generalized seizures. These were classified as scissors spasms, scratching seizures, and myoclonic jerks. They appeared after about three-quarters of an hour, in contrast to the latent period of two to three hours after intravenous injection. Seizures persisted for hours unless stopped with nembutal. Cerebrospinal fluid (CSF) was tested for acetylcholine (ACh), the minimum amount detectable during our experiments being 0.4 to 3.0 μgm. %. After intravenous injections of 1.5 mgm. per kgm. of DFP preceding convulsions produced by thujone, small amounts of ACh appeared in three out of four experiments. Intravenous injection of 5.0 mgm. per kgm. of DFP resulted in 3.0 μgm. % of ACh in the CSF. No ACh was found after the other procedures described.

Introduction

The production of epileptic seizures through excess accumulation of acetylcholine (ACh) in brain tissue would be of great interest. It is natural to seek one common mechanism that might mediate convulsive seizures of many different kinds. The acetylcholine–cholinesterase system naturally comes to mind. The possibility that ACh is responsible for normal synaptic transmission in the brain is supported by considerable evidence. This has been reviewed by Feldberg (16) and Nachmansohn (29).

Cholinesterase (ChE) activity is increased in tissue from focal epileptogenic cerebral cortex in humans and monkeys. This suggests that the rate of liberation of ACh in such tissue is greater than normal (34). Recent studies have demonstrated the presence of ACh in the cerebrospinal fluid (CSF) of humans who have frequent epileptic seizures (12). ACh may therefore have a significant role in the production of convulsions. We have tried to produce a chronically epileptic animal by injecting the anticholinesterase diisopropyl fluorophosphate (DFP) into the carotid artery of cats. We hoped to restrict the action of this potent chemical to the brain, or even to one hemisphere of the brain, and thus to avoid its systemic effects. Any passage of DFP into the general circulation could be determined by the blood ChE levels before and after injection of the drug.

In other experiments, DFP was injected into the cisterna magna, again with the object of restricting its action to the brain.

Intracisternal injections were also carried out with sodium fluoroacetate, a drug that is known to produce seizures in some animals when given systemically (9).

Finally, experiments were made to determine the presence of ACh in the CSF of animals following injection of DFP and fluoroacetate, and after seizures produced by metrazol and thujone and by electric shock.

Methods*

1. *Carotid Artery Injections*

Using aseptic precautions and with the test animals under nembutal anesthesia (32 mgm. per kgm. of body weight), DFP** was injected into the exposed common carotid artery of six animals in amounts varying from 0.9 to 1.8 mgm. per kgm. The DFP was in an $M/100$ solution in propylene glycol. Immediately before injection, the DFP solution was diluted to 10 ml. with normal saline. A seventh cat was given a solution of propylene glycol without DFP, as a control.

2. *Cisternal Injections*

Cats were anesthetized with sodium pentothal (20 to 25 mgm. per kgm. intravenously). The back of the head and neck were shaved and CSF was withdrawn from the cisterna magna and replaced by freshly prepared solutions of DFP or sodium fluoroacetate*** ranging from minimal to lethal doses and contained in 1 ml. of normal saline or bicarbonate–Ringer solution. Control injections of saline and bicarbonate–Ringer solution were made. A second cisternal puncture was made under nembutal anesthesia (22 mgm. per kgm.) from 15 min. to five hours after the first puncture, and CSF was removed for ACh assays. Control assays were done on CSF drawn prior to intracisternal injections in 12 experiments.

3. *Cholinesterase Determinations*

Venous blood from the antebrachial cephalic vein was collected before injection of DFP and 24 hr. thereafter. The ChE activity was measured with the Warburg apparatus by the method of Ammon (2) and Odom *et al.* (33) using ACh as substrate.

ChE activity was taken as the volume of carbon dioxide in cu. mm. produced in 60 min. by 1 ml. of undiluted enzyme preparation. Decrease in ChE activity after DFP injection was expressed as the percentage of the preinjection level. Compensation was made for autohydrolysis of the ACh and for barometric and temperature variations.

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* *Cats were used in all experiments.*
** *DFP was obtained from the Army Chemical Center, Maryland, U.S.A.*
*** *Sodium fluoroacetate was obtained from Dr. E. S. G. Barron through Dr. K. A. C. Elliott.*

The red cell ChE activity reflects better than does the plasma the activity of the brain ChE. At least this is true for the rat, rabbit, monkey, dog, and man (22, 24, 31) and probably also for the cat. Difficulty was experienced in the measurement of the cat red cell ChE because of the very low values found even before the injection of DFP. A suitable method was developed and was used in the later experiments.

The red cells were washed twice in bicarbonate–Ringer and then hemolyzed in 0.024 M sodium bicarbonate in a volume twice that of the whole blood from which they were taken. The "ghosts" were then homogenized by the instrument of Potter and Elvehjem (35) and 0.5 ml. of the homogenate was placed in the side arm of the vessel. The procedure was then the same as for plasma.

4. *Acetylcholine Assays*

The presence of ACh in the CSF was determined with the isolated ventricle of *Venus mercenaria* according to the method of Wait (39) and Tower and McEachern (38). Samples of CSF showing ACh were submitted to hydrolysis and tested again as further identification. To the CSF was added 0.1 N sodium hydroxide, the mixture was boiled momentarily, and then neutralized with 0.1 N hydrochloric acid.

ACh detectable in the original CSF varied from 0.4 to 10 μgm. %. Clam heart preparations sensitive to 3 μgm. % or less were used. CSF samples were preserved by the method of Tower (36).

5. *Metrazol and Thujone Seizures*

Metrazol (pentamethylentetrazol) and thujone were used to produce seizures by intravenous injection. An ampoule of 10% *metrazol* was diluted one-third in water, and an amount containing 17 mgm. per kgm. was injected rapidly. *Thujone* was prepared as a 1% emulsion in 6% gum acacia, and 0.7 ml. per kgm. of the emulsion was injected rapidly (21). Both drugs in these quantities produced violent convulsions lasting 15 to 45 sec. Thujone was also given following a previous injection of DFP. CSF for ACh determinations was removed 15 to 50 min. following the onset of seizures.

6. *Electric Shock*

Single a-c. shocks of 130 v., 0.3 sec., were given through bitemporal electrodes with a Rahm electroconvulsive therapy machine; d-c. shocks of 85 to 145 v., 2 to 9 sec., were given to other animals. CSF for ACh determinations was removed two to three hours following the shocks.

7. *Electroencephalograms*

Cortical potentials were recorded in two of the intracarotid DFP experiments. The cat was put to sleep with pentothal. Four steel electrodes, made from Kirschner wire, were sharpened at one end and insulated with

Tygon to the tip and were placed symmetrically into the skull. Bipolar recordings were taken with an ink-writing electroencephalograph whose frequency response was 0.5 to 40 cycles between the 30% attenuation points and whose over-all linear amplitude was 1.5 cm.

Results

1. *Carotid Artery Injections of DFP (see Table I)*

During the injection there was a series of prolonged twitches of the musculature about the incision. In the control only very slight twitches were seen. In two of the experiments the artery dilated to almost twice its size during

TABLE I

INTRACAROTID INJECTIONS OF DFP

No.	DFP, mgm./kgm.	Immediate effects		Later effects			% Normal ChE	
		Dilat. of artery	Muscle spasm	Ipsilateral salivation	Ipsilateral myosis and duration	Ipsilateral tremor	Plasma	RBC
8	0	No	Slight	No	No	No	100	—
2	0.9	No	No	Yes	18 days	No	24	—
4	0.9	Yes	Yes	No	No	Yes	35	32
5	1.35	Yes	Yes	Slight	24 hr.	Yes	14	15
6	1.35	No	Yes	Slight	2 hr.	Yes	15	20
7	1.63	No	Yes	Profuse	6 days	Yes	13	0
3*	1.80	No	Yes	Profuse	Intense	Yes	17	4

The animal in this experiment died in 24 hr.

the course of the injection. Salivation began a few minutes later, and, when the dose was relatively high, it lasted for several hours. In all but one there was a pronounced constriction of the pupil on the side of the injection. This ipsilateral myosis lasted from hours to days. Intermittent tremor of the limbs and trunk appeared half an hour after the injection and lasted for hours.

Plasma ChE levels were low 24 hr. after DFP, in proportion to the amount injected (Table I). Red cell ChE levels were not measured in all experiments but they too were reduced considerably after DFP injection. The drug had therefore passed through the brain and into the systemic circulation.

In two of the experiments electroencephalograms (E.E.G.'s) were made before and 30 hr. after the injection. In one, 1.63 mgm. per kgm. of DFP had been given and there were E.E.G. abnormalities consisting of rapid sharp waves that came only from the side of the injection (Fig. 1). The other animal, which had received 1.35 mgm. per kgm. of DFP, showed no E.E.G. changes at the time the record was made. No clinical seizures were observed at any time.
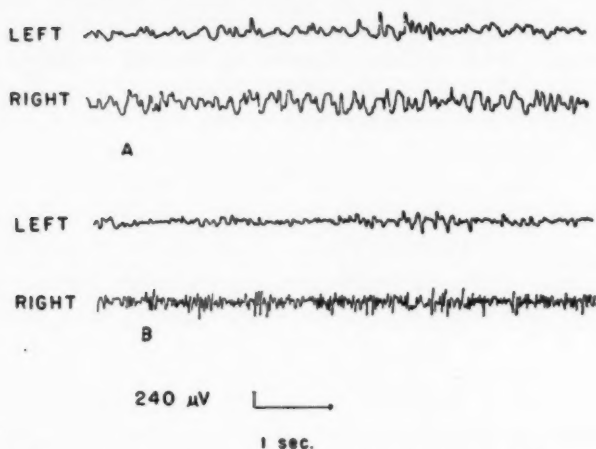
FIG. 1. *Electroencephalographic changes following injection of DFP. Experiment DFP-7, 1.63 mgm. per kgm. of DFP injected into right carotid artery. A, preinjection control. B, 30 hr. after injection.*

2. *Intracisternal Injections of DFP*

When a minimal dose was injected there was no immediate response. With a larger dose the depth of anesthesia was increased, as shown by loss of muscular tone and lack of response to stimuli. The duration of anesthesia was about three times that to be expected from the amount of pentothal used. This was very striking. Increased rate and depth of respiration followed within a few minutes in five of these experiments. Salivation, lachrymation, nystagmoid movements of the eyes, and twitching of the whiskers, all of short duration, were frequently seen in the first 10 min. Obviously the drug was affecting the brain-stem nuclei. When death occurred it was from respiratory failure. This was attributed to local action of the drug on the medulla.

Muscular tremor was noted within two hours of injection and lasted intermittently for one or two hours. The tremor resembled fasciculation.

When a forelimb of a conscious or even lightly anesthetized cat is pinched with forceps, or when the cornea is gently touched, there is usually a quick response. A diminished or absent response to this stimulation is described as sensory loss in Table II. It was a constant finding that persisted for many hours and sometimes for days.

Waddling gait with weakness of the hind limbs was observed in nine of the experiments after a sublethal dose of DFP. The animals showed ataxia upon awakening, and it was attributed to the anesthetic. However, the ataxia persisted for twice the usual time when DFP had been injected.

Plasma and red cell ChE dropped sharply from the preinjection levels and indicated that the DFP was absorbed into the general circulation.

TABLE II
INTRACISTERNAL INJECTIONS OF DFP
Summary of 23 experiments

No. of expts.	DFP, mgm./kgm.	Early effects (within 10 min.)	Later effects (within two hours)	% Normal ChE	
				Plasma	RBC
6	0-0.012	None	None	90-100	—
13	0.086-0.240	Deepened anesthesia Hyperpnea Salivation Lachrymation Nystagmoid eye movements Twitching of whiskers	Tremors Sensory loss Weakness of hind limbs Waddling gait	22-51	46-91
4	0.214-0.272	Deepened anesthesia Apnea and death by respiratory failure		8-24	—

3. *Intracisternal Injections of Sodium Fluoroacetate (see Table III)*

Increasing doses (up to 0.005 mgm. per kgm.) were given in six experiments but produced no definite effects. In the next 17 experiments 0.028 to 0.279 mgm. per kgm. was injected and definite effects were observed. These were of three main types and are here described as "scratching seizures", "scissors spasms" of the forelimbs, and myoclonic jerks. Usually two of the three appeared in the same animal and would continue for some hours until the animal was exhausted or the convulsions were stopped with nembutal.

"Scratching seizures" appeared in 11 of the 17 experiments listed in Table III, and the average time of onset was *three-quarters of an hour* after injection.

TABLE III
INTRACISTERNAL INJECTION OF SODIUM FLUOROACETATE
Summary of 23 experiments

No. of expts.	Sodium fluoroacetate, mgm./kgm.	Effects noted	Average time of onset after cisternal puncture
6	0.00002-0.005	Occasional twitch (not attributed to sodium fluoroacetate)	¼ hr.
17	0.028-0.270	1. "Scratching seizures" 2. "Scissors spasms" of forepaws 3. Myoclonic jerks	¾ hr. 1 hr. 2 hr.

At first the animal would briefly and accurately scratch the shaved area on the back of its neck with one or the other hind foot. This was repeated with increasing frequency, more and more violently, and with greater amplitude

but less accuracy. A sustained opisthotonic or lateral contortion of the trunk accompanied the scratching. Finally the movements became almost continuous, with perhaps a half-minute interval occurring every three minutes.

By the "scissors spasm" is meant a tonic crossed adduction spasm of the forelegs, usually with the neck forcibly flexed. This lasted for several seconds and frequently heralded a scratching seizure. The "scissors spasm" appeared on an average one hour after cisternal injection and was seen in 12 of the 17 experiments.

Myoclonic jerks of the extremities or trunk appeared in over half of the animals, usually after a latent period of two hours. Sometimes they appeared after the scratching seizures or scissors spasms were well established, and then the myoclonic jerks gradually replaced the other phenomena as the chief manifestation. In other animals myoclonic jerks alone were seen. Death, when it occurred, was by respiratory arrest.

Sodium fluoroacetate (0.090 to 0.270 mgm. per kgm.) was also given *by vein* to five cats. After a latent period of *two to three hours*, either myoclonic jerks or tonic seizures were seen, but none like the scissors spasm or the scratching seizures. The earlier occurrence of seizures following intracisternal injection, and also their different character, are worthy of note.

The peculiar scratching seizures were investigated further. Some cats with shaved necks tend to scratch themselves mildly and fleetingly, especially upon awakening from the effects of an anesthetic. This may occur irrespective of what has been injected into the cisterna. The scratching seizures observed by us, however, were characterized by their violence, their duration, and their apparently involuntary nature. In some cases the animals did not appear to be conscious. In one experiment, when the scratching seizures were well under way, the skin of the neck in the shaved area was infiltrated with 1% novocaine. This had no effect upon the seizures.

The convulsions induced by fluoroacetate were easily arrested by intra-peritoneal nembutal (22 mgm. per kgm.), which took effect after about 10 min. The animals were completely recovered by the next day.

Cisternal fluid was examined before and two to five hours after the injections in five experiments but no pleocytosis was found.

4. *Presence of Acetylcholine in Cerebrospinal Fluid*

Fifty-two samples of CSF were withdrawn from the cisterna magna of 20 cats before and/or after all types of procedures, and they were tested for ACh. Of these, 12 were controls, and the remaining 40 samples were taken at various times following the intracisternal or intravenous injection of the several drugs, and after electric shock. Amongst the controls, 0.4 μgm. % of ACh was found in two samples.

After intracisternal injections of DFP in high doses ACh was detected in the CSF in two out of five experiments. In one of these the original sample contained no ACh; four hours after injection (0.220 mgm. per kgm), 0.4

μgm. % of ACh was found.   In the other experiment the control sample contained 0.4 μgm. % ACh; 15 min. after injection (0.261 mgm. per kgm.) the amount had increased to 1.0 μgm. %.

In seven experiments convulsant doses of sodium fluoroacetate were injected into the cisterna magna.   CSF taken during the height of the seizures showed ACh in only one, and the control sample contained the same amount (0.4 μgm. %).

When a lethal dose of DFP by intravenous injection (5 mgm. per kgm.) was given, 3.0 μgm. % ACh was found in the CSF 10 min. later.   Sublethal doses in four experiments produced no detectable ACh in samples drawn 30 to 60 min. later.

ACh was not found in any of three experiments where sodium fluoroacetate was injected by vein in doses that produced seizures (0.200 to 0.270 mgm. per kgm.).   Neither did convulsions produced by metrazol (five experiments) or thujone (five experiments) increase ACh in the CSF to amounts that could be detected.

In four experiments convulsions were induced with thujone about one hour after DFP was injected intravenously, both drugs in amounts that had not produced ACh in detectable quantities in the CSF in previous experiments. In three of these experiments ACh was found to be present in the CSF 15 to 30 min. after the convulsion, in quantities ranging from 0.4 to 2.0 μgm. %.

Finally, ACh was not found in six experiments when seizures were produced by electric shock.   Perhaps the presence of an anticholinesterase is necessary.

In these experiments the presence of ACh was detected only when the threshold of sensitivity of the clam heart happened to be at the lowest in the series (0.4 μgm. %).   When there was no response to a particular sample of CSF the ACh content was reported negative.   There may have been ACh present, however, in amounts that could only be detected by a more sensitive test preparation.

Discussion

It is known that brain tissue can synthesize ACh through its choline-acetylase enzyme system (30).   ACh, or the anticholinesterase drugs eserine or neostigmine, can produce electrical evidence when applied locally to brain cortex in suitable concentration (8, 25, 27).   ACh and eserine can produce somatic effects when applied to the brain in various ways (25, 26).   ACh has also been recovered from the CSF of animals and humans after convulsive seizures (12, 37).   Atropine sometimes prevents these central cholinergic phenomena, but its inability to do so should not rule out a basic cholinergic mechanism.

Injections of DFP

DFP (18, 19, 40) when injected in various ways does affect the electro-encephalogram.   But it seems clear that, when injected in sublethal quantities into the carotid artery or the cisterna magna of cats, it does not produce a lasting convulsive effect.

Salivation and myosis following intracarotid injection of DFP in our experiments was probably a peripheral effect in the area of distribution of the external carotid artery. Brassfield *et al.* (4) report continuous salivation from injection of DFP into the isolated arterial supply of the submaxillary gland. Ipsilateral myosis was also obtained by Himwich and Freedman (20) following intracarotid injection.

The early effects following intracisternal injection of DFP are probably due to local action on centers in the medulla and pons near to the floor of the fourth ventricle. A sluggish response to pain after DFP has been described by Modell *et al.* (28) who injected this substance intravenously in cats. In our experiments the sensory loss was confined to the forelimbs and the cornea. We have no explanation for this, nor for the waddling gait and weakness of the hind limbs. They appear to be specific effects, however, and did not occur with control injections.

The strange thing is that DFP does not produce a greater effect on the brain. It is a lipoid-soluble substance, yet in our experiments, when it was injected so as to place it in close proximity to the brain it did not produce the results that might be expected. In fact, it passed through the brain and, in proportion to the injected amount, produced a lowering of plasma and red cell ChE in the systemic circulation.

Injections of Sodium Fluoroacetate

The action of this drug shows marked species variability (9). In dogs and guinea pigs it has an effect primarily on the central nervous system; in cats and rhesus monkeys it produces both a cardiac and a nervous system response; in rabbits and goats it affects chiefly the heart. By administration into the cisterna magna of cats we attempted to produce a more selective action on the brain.

Chenoweth and St. John (10, 11) obtained wave and spike forms of E.E.G. activity resembling that of petit mal when they injected fluoroacetate directly into the cerebrum of the rabbit through a burr hole in the skull. They infer (11) that fluoroacetate is incapable of traversing the blood–brain barrier in this animal. Disturbance of electrical activity in the brain appeared with a much shorter latent period following intracerebral injection. This agrees with the relatively short latent period (three-quarters of an hour) following intracisternal injection in our own experiments. When the drug is given intravenously there is a latent period of two to three hours before convulsant effects appear. The still considerable period that constantly precedes the cerebral action of fluoroacetate, even when given intracisternally, is unexplained.

Acetylcholine in Cerebrospinal Fluid

All available reports that ACh may be found in the CSF, together with our own results, are summarized in Table IV. Here it is seen that as more sensitive methods are used ACh is detected more frequently. It is possible

TABLE IV

ACETYLCHOLINE IN THE CEREBROSPINAL FLUID

Summary of reports in the literature

Author	Subject	Procedure	ACh found, μgm. %	Test object
Dikshit (15)	Cat	Vagal stimulation	1.0	Frog heart
Feldberg and Schriever (17)	Dog	1. Injection of eserine 2. Injection of adrenalin 3. Asphyxia	1.5 2.5 5.0	Cat's B.P. and frog heart
Adam *et al.* (1)	Cat Dog	Perfusion of ventricular system with eserinized fluid. Stimulation of hypothalamus	Positive; amt. not stated	Not stated
Chang *et al.* (7)	Dog	Vagal stimulation in eserinized animal	2.0–8.0	Leech muscle
Bornstein (3)	Cat Dog	Experimental brain trauma	2.7–9.0	Frog rectus abdominis
Tower (37)	Dog	Canine epilepsy induced by agenized flour	1.0 (2 expts.)	Clam heart
Nochimowski (32)	Human	Misc. neurological and psychiatric cases	10.0 (23 cases)	Leech muscle
Brecht (5)	Human	Meningitis; epilepsy	10^{-4}–2×10^{-2}	Frog lung
Brecht and Kummer (6)	Human	Psychiatric cases (schizophrenia and depression states)	10^{-4}–10^{-1}	Frog lung
Cone, Tower, and McEachern (12)	Human	1. Epilepsy 2. Brain trauma 3. Electric shock	0.1–5.0 (44 out of 56) 0.5–100 + (7 out of 20) 0.2 and 2.0 (2 out of 6)	Clam heart
Kelen and McEachern	Cat	1. Controls 2. Intravenous DFP 3. Intracisternal DFP 4. Intracisternal fluoroacetate 5. Intravenous metrazol or thujone or electric shock	0.4 (2 out of 12) 3.0 (1 out of 5) 0.4–1.0 (2 out of 5) 0.4 (1 out of 7) 0.0 (15 expts.)	Clam heart

that traces of ACh are always present in the CSF, even under normal circumstances, and that its significance will depend upon the *amount* found. Quantitative determination, by a suitably sensitive test object, may reveal that the amount of ACh varies with the kind of experimental procedure in animals, or with the nature of the illness in man. The clam heart (38, 39) will detect 0.04 μgm. % of ACh in the CSF in its best season. The positive results obtained by Tower and McEachern were largely in the range of 0.5 to 2.0 μgm. %, which was just beyond the threshold of many of our test objects

during the summer when these experiments were carried out. The frog lung preparation (5, 13, 14), which requires 8 to 10 ml. of fluid to be tested, will detect as little as 0.000001 μgm. % in the winter months. The latter method is however, very delicate and time-consuming. More studies are needed to establish the role of ACh in the production of seizures. Its appearance in CSF may only be an echo of events.

The effects of DFP are believed to be due to its activity as an anticholinesterase. Previous workers (7, 17) have found ACh in the CSF following the intravenous injection of eserine. Hence it is surprising that ACh is not found until lethal amounts of the more potent anticholinesterase, DFP, are given. It may be that too little DFP is taken up by the brain to reduce the brain cholinesterase to the necessary level.

These studies neither refute nor uphold the hypothesis that ACh has a key role in the production of seizures. Further analysis of the biochemical mechanism of seizures should be carried out on brain tissue itself.

References

1. ADAM, H. M., McKAIL, R. A., OBRADOR, S., and WILSON, W. C. J. Physiol. (London), 93 : 45P. 1938.
2. AMMON, R. Arch. ges. Physiol. (Pflügers), 233 : 486. 1933.
3. BORNSTEIN, M. B. J. Neurophysiol. 9 : 349. 1946.
4. BRASSFIELD, C. R., BIGGINS, C., and MUSSELMAN, M. M. Federation Proc. 6 : 80. 1947.
5. BRECHT, K. Klin. Wochschr. 19 : 1087. 1940.
6. BRECHT, K. and KUMMER, H. Klin. Wochschr. 22 : 741. 1943.
7. CHANG, H. C., HSIEH, W. M., LI, T. H., and LIM, R. K. S. Chinese J. Physiol. 13 : 153. 1938.
8. CHATFIELD, P. O. and DEMPSEY, E. W. Am. J. Physiol. 135 : 633. 1941.
9. CHENOWETH, M. B. and GILMAN, A. J. Pharmacol. Exptl. Therap. 87 : 90. 1946.
10. CHENOWETH, M. B. and ST. JOHN, E. F. J. Pharmacol. Exptl. Therap. 90 : 76. 1947.
11. CHENOWETH, M. B. and ST. JOHN, E. F. Research Pubs. Assoc. Research Nervous Mental Disease, 26 : 299. 1947.
12. CONE, W. V., TOWER, D. B., and McEACHERN, D. Trans. Am. Neurol. Assoc. 73 : 59. 1948.
13. CORSTEN, M. Arch. ges. Physiol. (Pflügers), 244 : 281. 1940.
14. DIKJSTRA, C. and NOVONS, A. K. M. Arch. intern. physiol. 49 : 257. 1939.
15. DIKSHIT, B. B. J. Physiol. (London), 80 : 409. 1933-1934.
16. FELDBERG, W. Physiol. Revs. 25 : 596. 1945.
17. FELDBERG, W. and SCHRIEVER, H. J. Physiol. (London), 86 : 277. 1936.
18. FREEDMAN, A. M., BALES, P. D., and HIMWICH, H. E. Trans. Am. Neurol. Assoc. 73 : 64. 1948.
19. GROB, D., HARVEY, A. M., LANGWORTHY, O. R., and LILIENTHAL, J. L., JR. Bull. Johns Hopkins Hosp. 81 : 257. 1947.
20. HIMWICH, H. E. and FREEDMAN, A. M. Federation Proc. 7 : 55. 1948.
21. KEITH, H. M. and STAVRAKY, G. W. Arch. Neurol. Psychiat. 34 : 1022. 1935.
22. KOELLE, G. B. and GILMAN, A. J. Pharmacol. Exptl. Therap. 87 : 421. 1946.
23. KREMER, M. Quart. J. Exptl. Physiol. 31 : 337. 1942.
24. MAZUR, A. and BODANSKY, O. J. Biol. Chem. 163 : 261. 1946.
25. MERRITT, H. H. and BRENNER, C. Arch. Neurol. Psychiat. 48 : 382. 1942.
26. MILLER, F. R. J. Physiol. (London), 91 : 212. 1937.
27. MILLER, F. R., STAVRAKY, G. W., and WOONTON, G. A. J. Neurophysiol. 3 : 131. 1940.
28. MODELL, W., KROP, S., HITCHCOCK, P., and RIKER, W. F. J. Pharmacol. Exptl. Therap. 87 : 400. 1946.

29. NACHMANSOHN, D.   *In* Vitamins and hormones, Vol. 3, *edited by* R. S. Harris and K. V. Thimann.   Academic Press, Inc., New York.   1945.
30. NACHMANSOHN, D. and MACHADO, A. L.   J. Neurophysiol. 6 : 397.   1943.
31. NACHMANSOHN, D. and ROTHENBERG, M. A.   J. Biol. Chem. 158 : 653.   1945.
32. NOCHIMOWSKI, C.   J. physiol. et path. gén. 35 : 746.   1937.
33. ODOM, G., RUSSEL, C. K., and McEACHERN, D.   Brain, 66 : 1.   1943.
34. POPE, A., MORRIS, A. A., JASPER, H., ELLIOTT, K. A. C., and PENFIELD, W.   Research Pubs. Assoc. Nervous Mental Disease, 26 : 218.   1947.
35. POTTER, V. R. and ELVEHJEM, C. A.   J. Biol. Chem. 114 : 495.   1936.
36. TOWER, D. B.   Can J. Research, E, 27 : 20.   1949.
37. TOWER, D. B.   Personal communication.
38. TOWER, D. B. and McEACHERN, D.   Can. J. Research, E, 26 : 183.   1948.
39. WAIT, R. B.   Biol. Bull. 85 : 79.   1943.
40. WESCOE, W. C., GREEN, R. E., McNAMARA, B. P., and KROPS, S.   J. Pharmacol. Exptl. Therap. 92 : 63.   1948.
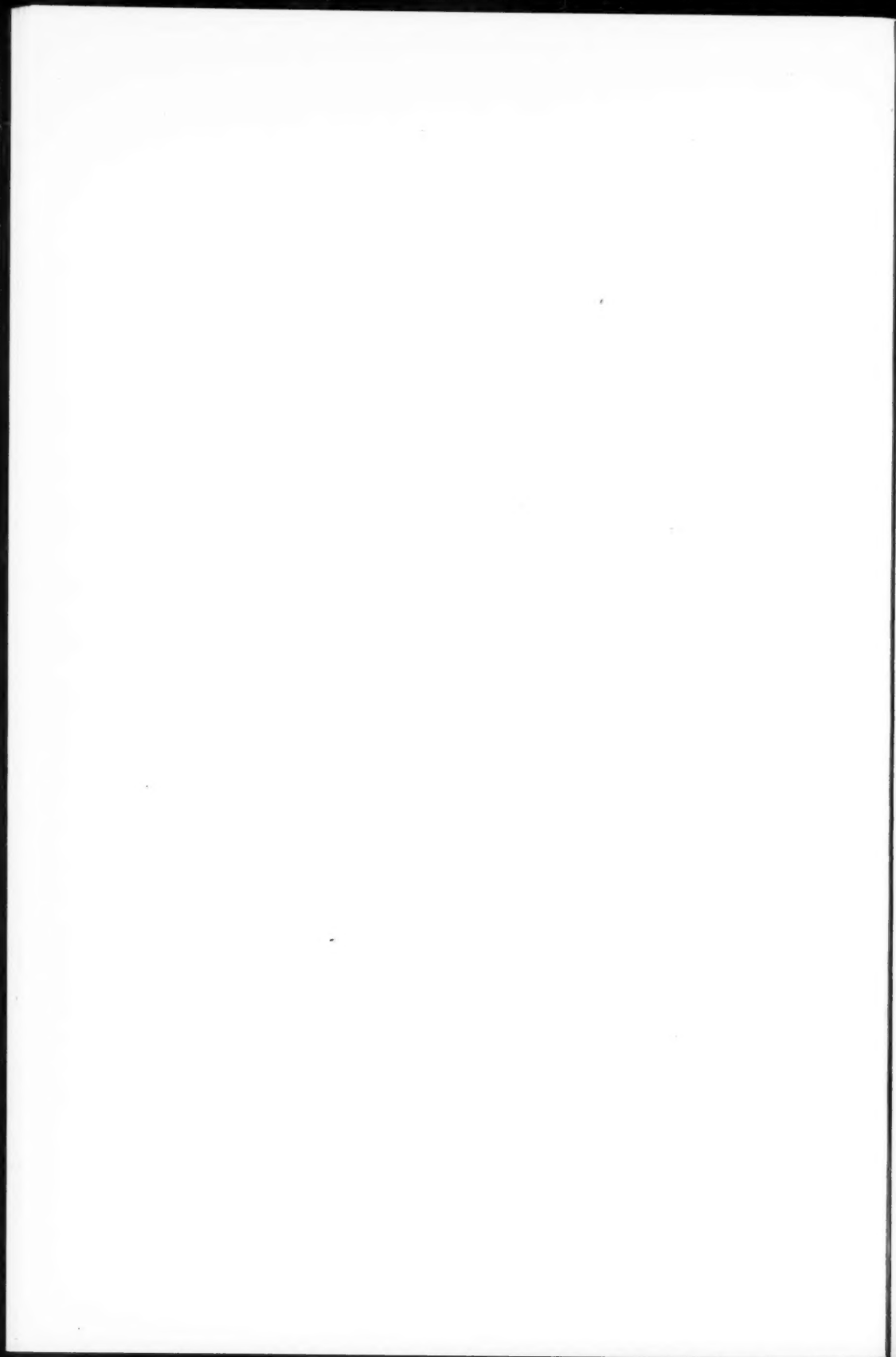
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